STUDIES

FROM THE

DEPARTMENT OF PATHOLOGY

OF THE

College of Physicians and Surgeons

COLUMBIA UNIVERSITY
N. Y.

VOL. XVII

1920

REPRINTS

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PREFACE

The published papers collected in this volume represent the results of investigations carried out in the Department of Pathology of the College of Physicians and Surgeons and in the Pathological Laboratory of the Presbyterian Hospital during the years 1918, 1919, and the first six months of 1920.

JAMES W. JOBLING, M. D.,
Director.

DEPARTMENT OF PATHOLOGY
COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY, NEW YORK
AUGUST, 1920

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EXAMINATION OF THE BONE-MARROW AT AUTOPSY WITH A METHOD OF OBTAINING SPECIMENS

ROBERT A. LAMBERT, M.D.

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It may be safely stated, I think, that routine examination of the bone-marrow at autopsy is done in very few institutions, and that one rarely sees sections of bone-marrow except from cases where a definite lesion was suspected during life; such, for example, as carcinoma with metastases to bones, pernicious anemia,

leukemia, etc. This is true even in laboratories where practically every other organ or tissue of the body is carefully investigated grossly and microscopically as a matter of routine. The general neglect of a tissue of such obvious importance can hardly be attributed to a failure to recognize its importance. It might possibly be suggested, that on account of the difficulty in interpreting pathological changes in the bone-marrow, its examination is hardly worth while; but the same might be said of the adrenal, hypophysis, or even the spleen (and, in my opinion, with greater justification) organs, which in many pathological laboratories are regularly sectioned and studied. After examining the bone-marrow from several hundred autopsies, I have no hesitancy in saying that definite and easily recognizable pathological changes occur more often in this tissue than in many other organs or tissues, to which far more attention is generally given. I hope to present a review of this material before the Society at another time.

I believe that the real explanation of the general disregard of the bone-marrow lies in the fact that its investigation requires some extra trouble, and that the result of the effort to obtain specimens is not always fruitful or satisfactory. Specimens containing bone require decalcification, which often spoils the material for subsequent microscopic study. In view of the difficulty in obtaining satisfactory pieces of bone-marrow with the methods ordinarily employed, I have thought it worth while to describe a method which an experience of two years has proved satisfactory in every way. I should explain that it was at Professor MacCallum's suggestion that an investigation of the bone-marrow was made a part of the autopsy routine at the Presbyterian Hospital, and that the method described here is a modification of one that he proposed.

A lantern slide illustration will show the autopsy incision which permits an examination of the marrow of the humerus. The incision has the added advantage of giving a very free exposure of the neck organs without entailing a cut in the skin of the neck, to which objection is often made, especially in

autopsies on women. The first part of the incision starts on the right shoulder or upper third of the right arm, passes upward, forward, and then downward, making a broad curve, the lower border of which crosses the sternum about the third rib; the cut ends on the opposite shoulder. A second median, vertical incision begins at the point where the first crosses the sternum and ends at the pelvis. Dissection of the flaps on one side exposes the upper third of the humerus. Two parallel saw cuts are then

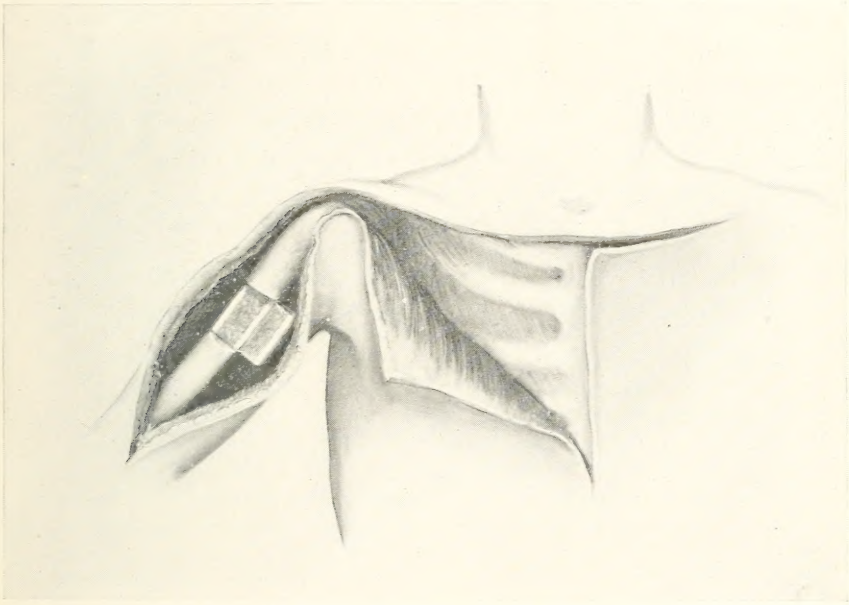

FIG. 1.

made; the first just below the surgical neck, the second only two inches below the first. In each, the saw is carried at least halfway through the bone. Then with hammer and chisel, the bone flap between the two saw cuts is lifted off, exposing the marrow cavity. Care should be exercised not to chisel deeper than the saw line, otherwise the bone will fracture. The marrow is viewed in situ; the color and consistence are noted; and then blocks of it are removed with a bone curette, which should be of a size just admitting insertion into the cavity. Though a very friable

tissue, bone-marrow can be taken out in small solid chunks. These are dropped immediately into small bottles containing fixing solution (Zenker's fluid and formaldehyde), and kept separate from the other tissues throughout the entire process of embedding. We find very convenient the ordinary fifteen c.c. bottles with wide mouth. After being well corked, these are dropped into the big jar containing the other organs from the autopsy, and the necessity for separate labeling is thus avoided. By using a small curette, pieces of bone-marrow can be secured from the head of the humerus, as well as from the shaft several cm. below the opening made. In the specimens obtained in this way, there are generally only a few spicules of bone, and decalcification is not necessary, especially if the tissue is fixed in Zenker's fluid, which tends to soften any small bits of bone that may be present.

Discussion:

DR. EWING: I think it is essential to examine the bone-marrow as a routine procedure. I always have done so and take as a rule a rib and vertebral body. It seems to me that if you ran that cut up to the head of the bone you would get a better idea of the changes in the bone-marrow which may be limited to the ends of the bones. Of course, the main difficulty with the study of bone-marrow is that you cannot examine all the bones in the body.

DR. LAMBERT: We usually do make the opening in the bone a little higher up than is indicated in the picture, so that it is possible with a curette to obtain specimens of marrow from the diaphysis of the bone. Dr. Ewing is probably right in pointing out that bone-marrow from more than one part of the skeleton should be examined, and I believe it would be well to take pieces from one of the flat bones, such as the vertebræ, as well as from the humerus or femur, as has been our custom. However, on the basis of the studies of the marrow in more than 150 cases, I am inclined to believe that bone-marrow changes are generally diffuse, and that an examination of even a single specimen from one long bone gives a fairly accurate idea as to what has been going on in the entire blood-forming apparatus.

DR. EWING: Is there any objection to this cut on the part of the undertaker?

DR. LAMBERT: We have never received any protests, either from the undertakers or from the relatives of the deceased. On the contrary, several undertakers have expressed their approval. I might explain that we are careful to leave the carotids intact, even when there is a thorough examination of the neck organs, and that to each carotid a string is attached, the free end of which passes out through the autopsy incision. This makes it an easy matter for the undertakers to find the vessels, and they seem particularly grateful to us for our attention to this point.

TISSUE CULTURES IN CANCER.[1]

Paper presented before the Second Pan American Scientific Congress, Washington, U. S. A., December 27, 1915–January 8, 1916.

By ROBERT A. LAMBERT,

epartment of Pathology, College of Physicians and Surgeons, Columbia University, New York City.

ABSTRACT.

The cultivation of tissues in vitro forms a very valuable addition to our methods of vestigating cancer problems. Factors may be introduced and controlled to an extent t possible in experiments upon the living animal. One may observe directly the vision of cells and follow succeeding generations of cells under variously modified nditions of life.

There are limitations to the method. Not every cancer cell or normal tissue can be ltivated, and when cultures are successful growth is by no means comparable to that served in cultures of bacteria. Some tissues merely survive. Human tissues offer ecial difficulties, although modifications in technique have enabled us to maintain active multiplication of human cells for more than a month. It is probable that owth may be kept up indefinitely.

The method of tissue cultures has been used by us chiefly in studying the nature of ncer immunity, and in carrying out comparative studies upon the biology of cancer lls and normal cells. Further evidence has been obtained to show that cancer immunity is not a serum immunity, that circulating antibodies for the cancer cell do not ist. Tumor cells grow actively in the plasma of animals which are naturally or tificially highly resistant to tumor inoculation. They even grow, as do also normal lls in the plasma of certain foreign species, except when such animals have been mmunized by suitable injections against the tissues of the species to which the tumor longs. In other words, cytotoxic immunity, in contrast to tumor immunity, is adily demonstrable in tissue cultures.

Interesting differences are observed in the behavior of cancer cells and normal lls in cultures, especially when the cells are subjected to the action of certain rmful agents. Cancer cells, particularly sarcoma, grow very actively in the primary ltures, but continued propagation through subcultures is difficult, often impossible. he reverse is true of normal connective tissue cells which become more active in bcultures. This observation is probably significant, indicating either that tumor lls are less hardy or that in cultures some necessary substance is not sufficiently pplied.

To some physical agents, such as cold, tumor cells (mouse and rat sarcomata) appear be more resistant than normal tissues. To other injurious agents, notably heat, e neoplastic cell is clearly less resistant. Recent experiments indicate that these sults apply also to human tissues. It is suggested that the problem of the therapy cancer may be profitably attacked by this method.

Efforts have been made to stimulate in various ways the growth in vitro of cells, th normal and malignant. Work in this direction is promising, and is undoubtedly greatest importance. The author has been unable to confirm some of the positive sults reported by others. Some substances which stimulate the growth of cells the body appear to be without effect in cultures.

Finally, it is perhaps interesting to note that normal cells grown for a long time tside the body do not develop as the result of their freedom from the restraint of e organism any tendency to malignant lawless growth. This is shown by the tirely negative results following the inoculation of subcultures actively growing of rmal connective tissue cells.

[1] The committee on publication regrets that, owing to the absence of the writer in South America, only this abstract of Dr. Lambert's paper can be printed.

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MALIGNANT NEUROBLASTOMA

ROBERT A. LAMBERT, M.D.

(From the Pathological Laboratory of the Presbyterian Hospital, New York)

The term "malignant neuroblastoma" has been applied in the last few years to a tumor occurring generally in infants or young children and arising from the undifferentiated tissue of the sympathetic nervous system. The location of the primary tumor is most often in the adrenals, less commonly in the retroperitoneum or mediastinum; but owing to the wide distribution of sympathetic nervous tissue, and the migratory power of its formative cells, the tumor may, theoretically, at least, have its origin almost anywhere in the body.

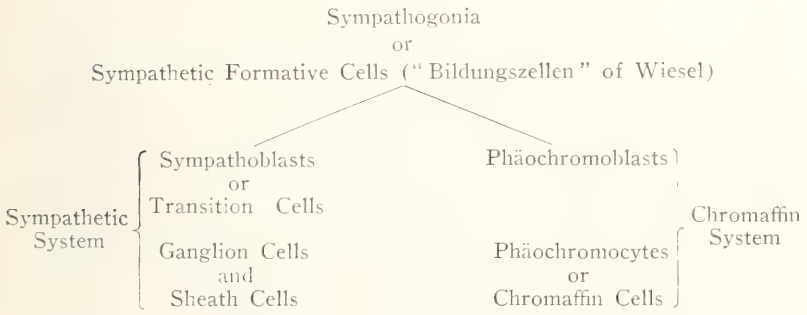
The true nature of this tumor was not generally recognized until a few years ago. In the eighties, nineties, and even as late as 1910, there were reported cases of lymphosarcoma, round-cell sarcoma, glioma, and gliosarcoma of the adrenals which, it is evident in the light of recent studies, were examples of neuroblastoma. In 1891, Marchand described a tumor of the adrenal and pointed out the resemblance of the cells to those of embryonic nerve tissue; it was not, however, until 1905 that the histogenesis of this group of tumors was definitely established. In that year Küster, a pupil of Ribbert, reported two tumors of the adrenal in children, which he interpreted as gliomata. Wiesel, who had made a special study of the development of the sympathetic nervous system, and especially of the origin of the adrenal medulla, through the invasion of the "anlage" of the adrenal cortex by formative cells of the sympathetic, called at-

tention to the similarity between the tissues composing Küster's tumors and these undifferentiated formative cells, or "Bildungszellen," pointing out that the morphology of the cells and the character of the fibrils in the neoplastic and embryonic tissues were practically identical. Wiesel's contention was not disputed, but tumors of the adrenal of this type were still reported under various names until 1910, when Wright published a brief but complete review of the question, a review which has secured for this tumor fairly general recognition. He collected from the literature eight cases which, from descriptions and illustrations, could be placed in the neuroblastoma group; and to this number he added four cases of his own. In his paper, Wright set down concisely the distinguishing histological characteristics of the tumor; these may be summarized as follows: The tumors are rich in cells, which may vary much in size and shape. They may be pervaded by connective tissue, the cells of which are arranged in more or less definite alveoli. The essential cells of the tumor are considered to be undifferentiated nerve cells, neurocytes or neuroblasts, for the following reasons: First, the cells, at least in places, are associated with delicate fibrils, often of considerable length, which do not stain like neuroglia, collagen, or fibroglia fibrils by Mallory's methods, and which are like the fibrils occurring in the "anlage" of the sympathetic nervous system; second, cells associated with these fibrils have the same morphology as the cells from which the sympathetic nervous system and the medulla of the adrenal develop, which are regarded by embryologists as arising from migrated primitive nerve cells. They are generally small, with round nuclei rich in chromatin, and have a relatively small or imperceptible amount of cytoplasm. Some of them may be pyriform in shape and their cytoplasm may be prolonged into filamentous processes like the fibrils referred to above; third, the cells and fibrils in places are arranged more or less like the neurocytes and their fibrils in the sympathetic nervous system at certain periods of its development and in the "anlage" of the adrenal. The fibrils may be arranged parallel to one another in bundles which are intimately associated with masses of cells, or they may be seen as a mesh-

work in the center of ball-like aggregations of cells to which the name "rosette" has been applied.

Since Wright's paper was published, at least eleven other cases of malignant neuroblastoma have been reported, and several excellent reviews of the subject of tumors of the sympathetic system have appeared. One of the best of these is by Wahl, in which there is a thorough discussion of all three of the well-recognized tumors originating from sympathetic nervous tissue, namely, *malignant neuroblastoma,* arising from the undifferentiated sympathetic formative cells, *ganglioneuroma,* from differentiated nerve fibers and ganglion cells, and *paraganglioma,* from chromaffin cells. The relations of these three tumors to one another and to the tissues of the sympathetic system out of which they develop are well shown in the following diagrams taken, with some modifications, from Wahl's paper.

CHART I



In the second diagram, in addition to the three well-recognized tumors emphasized by italics, the names of two other tumors are given, sympathoblastoma or transition-cell tumor, and phäochromoblastoma, a tumor made up of undifferentiated chromaffin cells. Only a single case of sympathoblastoma has been reported (Martius, 1913), and that was not a pure example, showing in places differentiated nerve cells and fibrils. Phäochromoblastoma is only a theoretical possibility, no such tumor having yet been described.

CHART II

Malignant Neuroblastoma (Landau)
Neurocytoma (Wright)
Ganglioma Embryonale Sympathicum (Pick)

Sympathoblastoma (Martius) — *Ganglioneuroma*

Phäochromoblastoma — *Paraganglioma* (Suzuki) or Chromaffin Cell Tumor

Of the three names proposed for the malignant tumor developing out of differentiated formative cells, malignant neuroblastoma, proposed by Landau, is to be preferred. The term "neurocytoma" should not be used for this tumor, since it has been given already by Marchand to a tumor of the cerebrospinal system made up of entirely undifferentiated cells without fibril formation. Pick's term, "ganglioma embryonale sympathicum," is inaccurate, as Wahl has pointed out, because it implies the presence of either differentiated or undifferentiated ganglion cells, whereas the formative cells of the tumor may give rise to either ganglion cells or chromaffin cells.

The writer's interest in tumors of the sympathetic system was aroused by the study of a case of malignant neuroblastoma which came to autopsy recently at the Presbyterian Hospital. The relative infrequency of this tumor is the chief excuse for presenting a report of it to the Society. Additional interest may be given the case, however, by the fact that it does present certain atypical features.

Clinical History.—Girl, aged five years, had diarrhea when eight months old, and measles at three years. Seven months ago she had an abscess of the neck which healed readily after surgical incision.

Present illness began four and a half months before admission to hospital, that is, six months before death, with severe cold, accompanied by cough and fever. After a few days in bed, the patient developed shifting pains in joints, abdominal pain after eating, and attacks of frontal headache every day or

two; she lost weight steadily with progressive weakness and anemia; had been continually in bed. Enlargement of axillary and cervical lymph-nodes was noticed six weeks before coming to the hospital, that is, three months after onset of illness.

Physical examination on admission showed marked enlargement of axillary, cervical, and inguinal lymph-nodes, prominent nodules on right frontal bone, right-sided exophthalmos, distended abdomen, enlargement of heart to left, and anemia.

The red blood cells numbered 2,400,000; the hemoglobin was 41 per cent. There were 6,000 leucocytes, with 72 per cent. of neutrophiles. There were no nucleated red cells. The Wassermann reaction was negative. Examination of the urine was negative (no Bence-Jones protein). An *x*-ray plate showed extensive rarefaction of pelvic bones, lumbar vertebræ, tibia, and skull.

The patient died after six weeks in the hospital. During this time there was irregular remittent fever, generally 100° to 102°, pulse 120 to 140. Weakness increased progressively with enlargement of the lymph-nodes, especially the axillary and cervical. A large abdominal mass could be palpated in the region of the umbilicus, and another about the rectum. Exophthalmos became pronounced in both eyes. A lymph-node was excised, and microscopic examination of this established the diagnosis of malignant neuroblastoma.

The autopsy was performed sixteen hours after death. The body was markedly emaciated and small for the age. Exophthalmos was pronounced in both eyes, most marked in the left. The glands of the neck, axilla, and inguinal region on both sides were greatly enlarged, but fairly discrete; the popliteal glands were palpable; those of the epitrochlear region were not. On the right frontal bone there was a rounded firm prominence, 5 cm. in breadth, over which the skin moved readily. Less porminent nodules could be felt over the entire vertex.

Thorax.—There was a small thymic fat pad, weighing about 15 gm., but little visible thymic tissue. There was a slight excess of fluid in both pleural cavities, with organized adhesions

FIG. 1. Neuroblastoma: Retroperitoneal tumor with involvement of peripancreatic, aortic, and pelvic lymph-nodes. Adrenals, kidneys, ureters, bladder, and rectum, partially surrounded but not invaded by tumor.

about the apex of the left lung. *Heart and lungs* showed nothing pathological except pallor of the cardiac muscle. After their removal, a mass of tumor tissue was seen in the posterior mediastinum, surrounding the descending aorta, and small nodules were firmly attached to the ribs in various places. These nodules extended into the ribs and were obviously metastases in the bone which had broken through the periosteum. The costal pleura over them was smooth.

Abdomen.—The peritoneal cavity contained about 100 c.c of clear yellow fluid. The liver extended 3 cm. below the costal margin; the kidneys and spleen appeared to be in normal positions. The stomach and pancreas were pushed forward by a large, firm, nodular mass in the retroperitoneum (Fig. 1). The mass had no definite outline, but extended down along the aorta, and was continuous with the enlarged iliac and inguinal nodes. The mesenteric nodes were small, none exceeding 0.5 cm. in diameter.

The *liver* weighed 700 gm. and like all the other organs was pale, almost white when the blood was washed off. The lobules were faintly seen, but were of normal size; there were no tumor nodules.

The *spleen* weighed 75 gm. The Malpighian bodies were distinctly seen as white, opaque dots scattered uniformly through the pulp; there was no gross evidence of tumor.

The left *adrenal* could be easily dissected from the retroperitoneal mass; it was quite thin, but the cortex and medulla were distinctly outlined. The right *adrenal* was partially surrounded by tumor, but not infiltrated by it (Fig. 2). Like the left, it was very thin, as though compressed by the adjacent tumor mass.

The *kidneys, bladder, uterus, adnexa, esophagus, stomach,* and *intestine* showed nothing pathological.

In opening the *skull* the saw line was carried through the prominence on the right forehead. This was found to be tumor, evidently a metastatic nodule in the frontal bone. Similar smaller metastases were present in other portions of the skull. The metastases in the frontal bones had extended through the

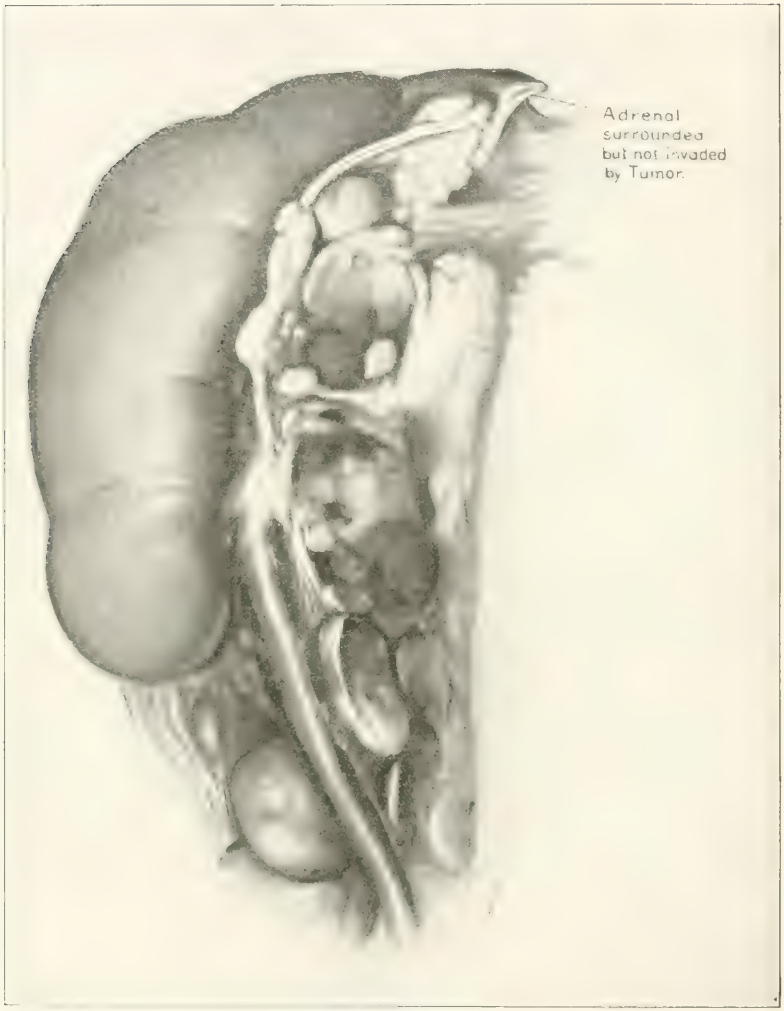


FIG. 2. Right kidney, adrenal, and retroperitoneal tumor. Neighboring lymph-nodes are replaced by tumor, but kidney and adrenal are not invaded.

dura and invaded both frontal lobes. There was a similar invasion of the right parietal lobe. A mass of tumor projected into the cranium from each orbital fossa. Multiple sections through the *brain* showed no metastases except those which had invaded it through the skull.

The size of the main abdominal tumor with its anatomical relations is very well shown in the first cut. The tumor had no limiting capsule and had spread widely through the retroperitoneal tissues. There was, however, no macroscopic infiltration of any of the adjacent viscera—pancreas, spleen, liver, adrenals, or kidneys. A large part of the tumor mass was made up of big, partially fused, retroperitoneal lymph-nodes which were entirely occupied by neoplastic tissue. The iliac and inguinal nodes were similarly involved, but were fairly discrete with little or no evidence of invasion of their capsules. This was true also of the axillary and cervical nodes, some of which measured 5 to 6 cm. in diameter. The tracheal nodes were smaller, but in places their capsules had been invaded with an extension of the tumor to the adjacent muscles. The glands at the hilum of the lung were not involved. There was, however, a continuous stretch of tumor along the entire length of the aorta, in which the capsule of a node could now and then be made out. The psoas muscles were extensively infiltrated. In the deeper portion of the pelvis, around the rectum, there was a big mass of tumor which was continuous with the pelvic bones. It was difficult to make out which way the tumor was growing, whether from the bones into the pelvis or vice versa.

The character of the tumor tissue was very much the same everywhere. It was firm, but not hard, and moderately friable. The color in most places was a uniform gray, and the tissue was semi-translucent. There was only an occasional fibrous septum, and no macroscopic foci of necrosis or softening. There were a few small hemorrhages in the cervical and axillary nodes; and in the retroperitoneal tumor several extensive hemorrhagic areas were present. These were seen also in the metastases in the skull bones.

It is not possible to say with absolute certainty just where the tumor originated, but the greater size of the retroperitoneal growth makes it probable that the tumor began there.

Microscopical Examination.—The tumor showed every-

where the same histological features; and the description of the inguinal node removed during life applies equally well to the tumor found at autopsy:

The growth was composed of cells relatively small in size, with round, chromatin-rich nuclei and scanty cytoplasm. There was a tendency toward an alveolar arrangement of the cells, which was quite pronounced in some sections. In places there were aggregations of cells arranged in circles with a central,

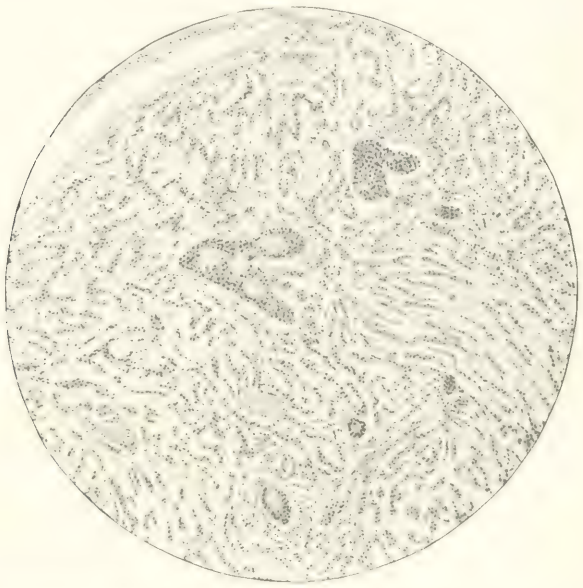


FIG. 3. Neuroblastoma: Low power drawing showing alveolar arrangement of cells and rosettes.

pink-staining tangle of fine fibrils, identical in every respect with the rosettes described by Wright and others. The stroma of the tumor was quite loose and delicate, but was everywhere distinctly seen. Rosette formation was a more striking feature of some of the large retroperitoneal nodes than of the inguinal node (Fig. 3). Indeed, in some of the sections almost all the tumor cells were thus arranged. In such places the stroma was very scanty. In a section of one gland, several areas of necrosis were

found, but in general both necrosis and hemorrhage were only exceptionally encountered.

Macroscopic metastases were noted only in the lymphatic and osseous systems, but microscopically they could be demonstrated, also, in the ovary, liver, spleen, and adrenals. In the ovary, there was a small but well-organized tumor nodule occupying less than a low power microscopic field. In the spleen,

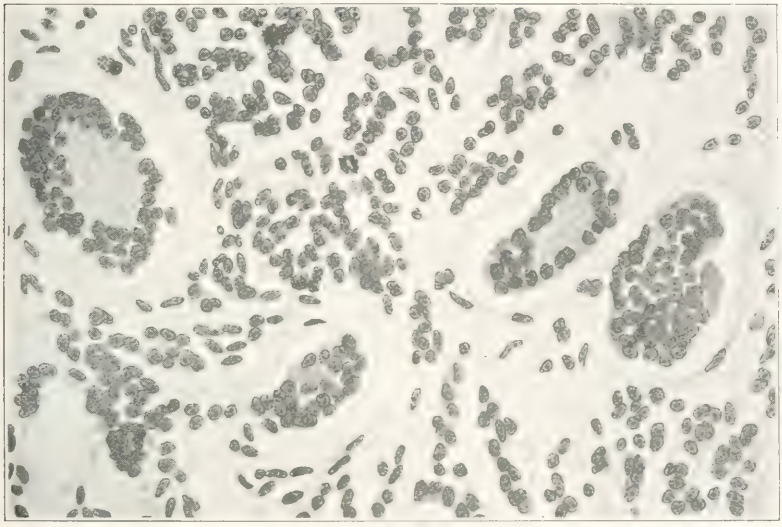


FIG. 4. Neuroblastoma: High power drawing showing morphology of cells, formation of rosettes with central mass of fibrils, and delicate connective tissue stroma.

there were no circumscribed tumor foci, but scattered everywhere through the pulp were small aggregations of tumor cells, arranged generally in rounded clumps or rosettes, which appeared to lie in distended sinuses without any supporting stroma (Fig. 4). The lymphoid follicles were nowhere invaded. In the liver, two types of foreign cells were seen: first, cells resembling lymphocytes with deep-staining nuclei and scanty cytoplasm lying between the cords of liver cells. These were few in number and never more than five or six together; they were prac-

tically identical in appearance with the blood-forming elements generally seen in the livers of congenital syphilitic infants, and represent, no doubt, an effort to extramedullary blood formation, demand for which was aroused through the extensive replacement of the bone-marrow by tumor. The cells of the second type, seen both in the liver and adrenal, were distinctly neoplastic. They lay free in the capillaries; and the groups were too small for any arrangement into ball-like aggregations or rosettes.

Specific neuroglia and fibroglia stains were applied to sections showing the most abundant fibril formation, but the results were uniformly negative. This is in accord with the experience of Wright and others.

FIG. 5. Neuroblastoma: Metastasis in spleen. Note discrete groups of tumor cells growing in sinuses of pulp, with tendency to rosette formation.

Anatomical Diagnosis.—Neuroblastoma, arising in retroperitoneum; metastases in iliac, inguinal, mediastinal, axillary, and cervical lymph-nodes, spleen, ovary, and osseous system, especially pelvic bones, vertebræ and skull; groups of cells free in

capillaries of adrenal and liver; replacement of bone-marrow by tumor with extramedullary blood-formation in liver; anemia.

SUMMARY

This case presents all of the distinguishing histological features of the malignant neuroblastomata. The tumor cells are quite characteristic in their morphology and in the tendency toward an arrangement into alveoli and rosettes. There is abundant formation of fibrils which do not give the specific neuroglia or fibroglia standing reactions.

In the location of the primary tumor and in the distribution of metastases the case is, however, not so typical. The accompanying list of previously reported neuroblastomata shows that the primary tumor was found most often in the adrenals, which in our case were uninvolved, except for the presence of small groups of cells free in the capillaries.

It is also exceptional, according to this list, for the liver to be spared in cases where metastases are present, gross nodules being found in fourteen out of sixteen cases. In our case there were no macroscopic metastases in the liver, and microscopically only a few cells in the blood-vessels were found, as in the adrenals.

The present case is the first to be reported in which metastases were found in the spleen and ovary. These were not recognizable in the gross specimens and were found only in the routine microscopical examination of organs. It is quite possible that similar small metastases may have been overlooked in some of the previously reported cases where complete microscopic studies were not made.

Widespread metastasis in the lymphatic system and bones, especially the skull, as found in the present autopsy, appears to be a characteristic feature of the more malignant types of neuroblastoma. The seven cases of primary sarcoma of the adrenal with metastasis to the skull, reported by Hutchinson, very probably belong to this group.

Cases of Malignant Neuroblastoma

Author, Date	Age	Sex	Location	Metastases	Diagnosis
Dalton, 1885.	6 weeks	M.	Left adrenal	Liver	Lymphosarcoma
Marchand, 1891...	7 months	F.	Right adrenal	None	Neurocytoma?
Amberg, 1904 ...	2 months	F.	Left kidney	Liver, right adrenal?	Malignant tumor of adrenal medulla
Fruck, 1904..	14 months	F.	Right adrenal	Liver, pancreas, skull, lymph gland	Hemorrhagic round-cell sarcoma
Richards, 1905....	2 weeks	M.	Left adrenal	Liver, lymph gland	Small round-cell sarcoma
Küster, 1905,					
Case I..	14 weeks	?	Right adrenal	Liver, left adrenal	Glioma
Case II.. ..	34 years	M.	Left adrenal	None	Glioma
Lapointe & Lecene 1907 ..	19 months	F.	Left adrenal	Lymph gland, mediastinum	Glioma
Alezais & Peyron, 1907.....	6 years	M.	Between rectum and sacrum	None	Parasympathoma
Schilder, 1909.....	7 days	F.	Right kidney attached to 2d left vertebra	None	Malignant glioma
Hecht, 909..	9 years	F.	Retroperitoneum	Adrenal, liver, lymph gland, duodenum	Lympho- or glio-sarcoma
Tileston & Wolbach, 1910	16 months	M.	Between right kidney and liver	Bones and lymph gland	Lymphoma?
Wright, 1910,					
Case I.....	1 day	M.	Both adrenals	?	Neurocytoma or neuroblastoma
Case II..	Adult	M.	Hilum of lung	Liver	do.
Case III	?	?	Cerebellum	None?	do.
Case IV.	16 months	F.	Multiple, retroperitoneum, skull	Mediastinum, liver, pelvis?	do.
Pick, 1912	23 years	F.	Uterus and broad ligament	Liver and lymph gland	Ganglioma embryonale sympathicum
Landau, 1913,					
Case I...	8 months	F.	Retroperitoneum	Liver	Malignant neuroblastoma
Case II.....	2½ years	F.	Right adrenal	Liver, lymph gland, kidney bones	Malignant neuroblastoma
Case III	1 day	F.	Left adrenal, right adrenal	Liver, lymph, gland	Malignant neuroblastoma
Symmers, 1913....	44 years	M.	Scapular region	None	Neuroblastoma
Anitschkow, 1913..	4 months	F.	Retroperitoneum	Infiltration into spinal canal	Malignant neuroblastoma
Dunn, 1914..	14 years	M.	Right adrenal	None	Neuroblastoma
Glomset, 1915 ..	2 years	M.	Right adrenal	Liver, lymph gland?	Neuroblastoma

Cases of Malignant Neuroblastoma—continued

Author, Date	Age	Sex	Location	Metastases	Diagnosis
Harbitz, 1915,					
Case I	3 years	?	In front of sacrum	None	Neuroblastoma
Case II	6½ years	?	Left adrenal	Liver	Neuroblastoma
Case III	Fetus	M.	Sacral region	?	Neuroblastoma

The cases of primary sarcoma of the adrenal reported by Parker, De Ruyter, Heaton, and Hutchinson probably belong here. They have not been included, however, because of incomplete data.

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Discussion:

Dr. Ewing: This case is peculiar in that it does not involve the adrenals. It is limited to the lymphatic system apparently, and in several respects it is different from the majority of adrenal neuroblastomata which tend to involve the liver. It might be well to add to the evidence already presented the results of specific stains, to determine whether definite fibrils can be demonstrated. I am inclined to think that neuroblastoma is not so rare as has been reported. I do not know whether Dr. Lambert has seen Dr. Symmers's case. We have another tumor in our laboratory which we have classified as a neuroblastoma, and I imagine that in the services of children's hospitals the country over, malignant tumors of this sort are still occasionally escaping recognition. I believe that Wright or Tileston and Wolbach have collected a number of cases which had been wrongly interpreted. It is probably true that certain tumors in children reported as lymphosarcomata are types of neuroblastoma of the adrenals. One of these groups, forming quite a series, was described by Pepper and Stengel some years ago.

Since a tumor of this nature may produce round cells, I think it wise that lymphosarcomata in children should in general be regarded with suspicion unless they are quite characteristic in distribution. Of course, with many indifferent round-cell tumors it may be impossible to recognize the histogenesis, but in my opinion, lymphosarcoma is the last diagnosis which should be made in children.

Dr. Moschcowitz: In connection with Dr. Ewing's suggestion that these tumors are probably more frequent than is generally supposed, I recall seeing abroad tumors demonstrated as gliomata, in which rosettes of an appearance identical with that described by Dr. Lambert were present. They are known as "Winterstein's rosettes." In all probability some of these tumors were neuroblastomata.

Dr. Lambert: This case is exceptional, though not unique, in not having its origin in the adrenal and in not showing extensive metastases in the liver. Among the twenty-seven cases which I was able to collect from the literature, there were eleven in which the adrenal was uninvolved; in four of these, the primary tumor was in the retroperitoneum, as in my case. Regarding the incidence of these tumors, I am inclined to disagree with Wright, who having seen four in one year, believes that the tumor is not an uncommon one, but is often overlooked. The growth presents such a striking clinical and pathological picture that it is difficult to understand how, in children especially, it could be overlooked or confused with any other type of neoplasm. Furthermore, several pathologists, including Symmers and myself, who have looked over a large number of tumors in several institutions, have been unable to find any other cases than those reported.

In making a diagnosis of neuroblastoma, special stains are of value only in a negative way, as I pointed out in my paper. Fibrils are abundant in most of the cases. They do not stain, of course, as neuroglia or fibroglia fibrils. Herxheimer, I believe, was able to show that they reacted as nerve fibers with the Bielschowsky silver method, which I have not used.

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N.S., Vol. XVIII No. 1–5, January–May, 1918.]

ECHINOCOCCUS CYSTS IN A MONKEY

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The specimen consists of a *Macacus rhesus* monkey, which died in the Bronx Zoölogical Garden. It was sent to Professor Huntington for anatomical study, but, finding a striking pathological condition present, he kindly turned the animal over to me. The abdomen was enormously enlarged, and full of cysts. These were attached to the peritoneum everywhere, but especially over the omentum, mesentery, and lower surface of the liver. Only the capsule of the liver was involved, there being no cysts anywhere in the substance of the organ. There were, also, no cysts in the spleen, kidneys, or thoracic organs. The cysts grossly resembled in every way those which are so commonly found in pigs, and were not different from the multilocular cysts of the liver occasionally encountered in man. The larger cysts contained daughter-cysts, and inside were the so-called granddaughter cysts. Microscopic examination of the fluid in the cysts revealed numerous scolices, which in the fresh state were seen to be definitely motile. Adherent to the inner walls of the sacs, the scolices could be seen with the naked eye as tiny, yellow specks. After autopsy the specimen was placed in a very cold ice-box overnight, and on the following morning, some of the cysts were fed to two dogs. These were kept for six weeks and then killed. In the feces of these dogs, during life and after death, we failed to find any ova of *Tænia echinococcus,* and a careful examination of the gut, at autopsy, revealed no worms. It is possible that the animals were not kept long enough, since some investigators have found that the worms do not develop before eight or nine weeks. It is also possible that these particular dogs were not favorable hosts, although it has been noted that the percentage of dogs which can not be infected is small. The fluid in the cysts was partially frozen in the cold ice-box, so that the

chilling of the worms may have been a factor in preventing infection.

It may be stated that echinococcus infection in monkeys is not rare, especially among those which have been fed on fresh meat in zoölogical gardens.

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A SIMPLE AND INEXPENSIVE METHOD OF PREPARING COLORED LANTERN SLIDES FOR TEACHING

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In teaching the course in pathology at the College of Physicians and Surgeons during the past few years, a projection apparatus, or epidiascope, has been used for demonstrating microscopic sections, photographs, drawings, etc. Recently, owing to structural alterations in the building, it was found impossible to darken the classroom sufficiently for satisfactory projection. We therefore had to resort to the use of lantern slides which, as you know, may be shown in a semi-darkened room. It occurred to us, however, that students, accustomed to stained histological preparations and colored illustrations, would not find the black and white lantern slide a satisfactory substitute for the projected stained section or colored picture. We therefore decided to use colored lantern slides, provided a simple and inexpensive method of preparing them could be devised. There are two types of colored lantern slides in common use, Lumière plates, and hand-colored slides. The preparation of Lumière plates was not attempted on account of the expense, and the recognized difficulties and unsatisfactory results of the method. The cost of hand-colored slides also appeared prohibitive, since the people who do this work as a business make a charge of a dollar to a dollar and a half per slide. We needed four hundred or five hundred slides, for which the cost, therefore, would have been around five hundred dollars. After some little experimentation, we found that lantern slides could be colored easily in the laboratory at small expense. The two methods, which have proved simple and practicable, can be described in few words.

The first method is practically the same as that ordinarily employed, except for a few short cuts and modifications. The

slide is printed light, but with as sharp definition as possible. Over this light positive, the desired colors are laid on with a fine brush. Japanese water colors are used. In illustrating microscopic sections, where generally a hematoxylin-eosin effect is aimed at, it has been found unnecessary to color the nuclei blue, —a tedious matter in low power illustrations,—since the difference between blue and the original black is not very noticeable. Cytoplasm, connective tissue, and muscle are colored pink. In many instances, a tone of pink applied rapidly over the entire slide produces the desired effect. To make a colored lantern slide from a colored drawing sometimes offers difficulty, since certain colors, particularly reds and yellows, come out very dark in the print, making it difficult or impossible to lay on subsequently the proper color. This difficulty has been overcome by touching up the negative so as to get a light positive, that may be easily colored. There are other small details which any intelligent laboratory assistant with good color sense will easily work out for himself. Illustrations of gross specimens are more easily colored than those of microscopic preparations, since there is generally less detail, and oftentimes a single tone over the entire picture will give a very pleasing effect. The shading in the original black and white gives the proper variations in depth of color. In this way, an ordinary uncolored slide, which may mean little to the students, can be converted into an impressive colored illustration in two or three minutes with little or no expense.

The second method consists in drawing colored pictures directly on the unexposed lantern slide plate. The plate is dipped into the fixing bath without previous exposure or development and then allowed to dry. On the film side of the glass, water color drawings can be made almost as easily and as rapidly as on paper. Such drawings project with great clearness and brilliancy, owing to the fact that practically no light is absorbed. Furthermore, the expense of photographing and printing is avoided. It may be explained that drawings can not be made on plain glass, and that something in the nature of a gelatin film over the glass is necessary. Such a film may be put on in the

laboratory, but we have found it advisable to use the ready prepared plates as indicated.

The first method has been used where we had on hand the illustrations which we wanted reproduced; the second has been used for illustrating microscopic preparations of which drawings and photographs have not been made, or for copying illustrations which can not be easily photographed.

Discussion:

Dr. Wood: I think Dr. Lambert is right when he says that color adds a great deal to teaching medical students by means of lantern slides, either of sections or gross material. We are never accustomed to black and white for microscopic sections, and our diagnosis depends very largely on our familiarity with stains. The method of coloring slides is a very old one, as Dr. Lambert says. To attempt projection of Lumière plates is a waste of time, for if you do have a good plate, it is too dark for use. Only one-tenth of the incident light passes through the slide. The Paget plates pass about fifty per cent., but they are technically difficult to make. There is a new process brought out by the Eastman Company which is not yet perfected. It promises well, but it is still difficult and uncertain. As far as I am concerned, I think the simplest way is to have the ordinary lantern slide hand colored.

Dr. Larkin: I think we all like to tell of our experiences in color photography. I thoroughly agree with Dr. Wood and all the other photographers who spend time on this worthy cause that our processes are far from perfect. I have something like one thousand Lumière plates covering many subjects, and though they are beautiful to look at through the bright sunlight, they are very disappointing when thrown on the screen. No matter how powerful the light is, poor results follow. This renders the Lumière plate unsuitable for teaching purposes. I have used a plate made by Fitzsimons of 75 Fifth Avenue. Fitzsimons maintains that only his developer should be used, and my own photographer, using Fitzsimons' developer, obtains more definition than when using the one advised by the Lumière people. With the plate which Fitzsimons has put out, we get very high color differentiation. One difficulty, however, is that when you put it into the lantern there is more or less of a haze over the picture.

Dr. Ewing: This is a very important subject because it deals with the methods of teaching medicine. At the next meeting of the American Medical College Association the main topic will be the discussion of pedagogical methods in medicine, and I feel that schools all over the country ought to investigate these methods, and standardize them as much as possible. It seems to me in this particular field there is an opportunity for great improvement in the material available in many schools in this country. There are sixty-two medical schools in the Medical College Association, and I am quite certain that not all of them have good material to demonstrate gross

and microscopical lesions. Now if it were possible for one firm to make one or two or three thousand lantern slides of gross and microscopical preparations it would be a very important addition to the material for teaching. Among other advantages, it might obviate certain idiosyncrasies which are apt to crop out from time to time in our teaching. Whether there is a commercial process which will produce such color plates for a small sum of money is questionable, but I think they could be cheaply produced by a technician employed by the College Association. Personally, I do not use colored lantern slides in teaching. I think my work falls down perhaps on that account. If you have over one hundred students you cannot give them all the desirable personal attention, and as we have always had small classes, I have limited myself to gross and microscopical material, a method which I think is distinctly better than photographs. When photomicrography first came out I was very conservative about its use, but the more you cater to the artistic color sense of an audience, the more they are interested. Yet there is no doubt that medical audiences are now pretty keen in interpreting ordinary photomicrographs, and I think they rather look forward to seeing them at a lecture, but for the teaching of medical students there is an advantage in colored slides.

Dr. Lambert: It has been very interesting and instructive to hear of the experiences of Dr. Wood, Dr. Larkin, and Dr. Ewing. I have learned quite a lot from them, especially what not to attempt in the way of color photography. In regard to what Dr. Ewing said about the relative value of microscopic sections and lantern slides in teaching, I should like to explain that in our teaching we use both lantern slides and sections. We find it difficult to demonstrate to each student all the things that he should see under the microscope, so that before or after they have studied the sections, a lantern slide demonstration is given by means of which the more important features can be pointed out to the class as a whole.

Relationship of the Leukocyte Count and Bone Marrow Changes in Acute Lobar Pneumonia

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RELATIONSHIP OF THE LEUKOCYTE COUNT AND BONE MARROW CHANGES IN ACUTE LOBAR PNEUMONIA

S. S. Samuels and Robert A. Lambert

From the Pathological Laboratory of the Presbyterian Hospital, New York

It is well recognized that the leukocyte count in lobar pneumonia may vary within wide limits. The majority of the cases which end fatally show either a very high or a relatively low count, while those with favorable outcome most often have counts between these extremes.[1] These points are brought out rather strikingly in the accompanying chart, showing a comparison of the leukocyte counts in 26 fatal and an equal number of recovered cases of acute lobar penumonia.

The cases with persistently low leukocyte counts may be considered first. At least two explanations for these relatively low counts have been suggested: (1) The bone marrow fails to react, either as the result of some previous injury (chronic alcoholism, for example), or on account of a paralysis of the blood-forming elements from overstimulation induced by the pneumococcus infection itself. (2) A rapid spread of the pneumococcus process results in the withdrawal of leukocytes from the blood faster than they are thrown into the circulation from the bone marrow, so that the number of circulating leukocytes may be normal or only slightly increased, in spite of the fact that the output of the bone marrow factory is far above normal. Still another possibility must be conceived, namely, that leukocytes may be formed in some other organ than the bone marrow, for example, the spleen. F. A. Evans,[2] working in this laboratory, pointed out several years ago that the gray, acute splenic tumor of lobar pneumonia contained large numbers of cells of the myeloid series, as indicated by the oxidase reaction, and suggested that the spleen might be the source of certain of the cells of the pneumonic exudate.

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[1] Osler: Principles and Practice of Medicine, Ed. 8, p. 87.
[2] Bull. Johns Hopkins Hosp., 1916, 27, p. 356.

The first explanation for the leukocyte count, namely, that the bone marrow fails to react, has been discussed by Muir, Longcope, Dickson, and others. In most of these papers the subject of acute lobar pneumonia has been considered secondarily, in connection with types of acute, nonsuppurative infections.

Muir,[3] in discussing the reaction of the bone marrow and other leukocyte-forming tissues in infection, found that the marrow pictures in his cases of acute pneumonia varied considerably. These variations he attributed to such factors as the age of the patient, the hyperplasia being less marked in late years; to the duration of the disease; to the severity of the toxemia, and to possible unknown causes such as the resisting power of the individual. He ascribed the phenomenon of depletion of adult polymorphonuclear leukocytes from the marrow to an emigration of these cells from the marrow just before death, probably in the agonal stage.

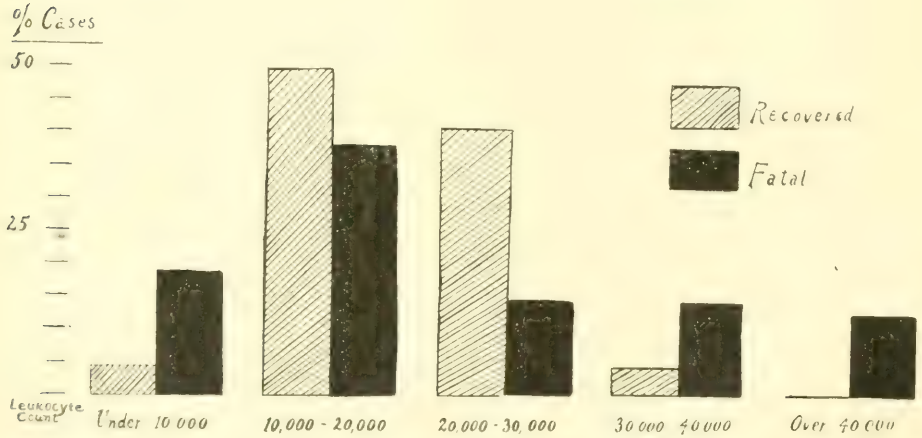

Chart 1.—Comparison of the leukocyte counts in 26 fatal and an equal number of recovered cases of acute lobar pneumonia.

The latter point was investigated experimentally by Longcope.[4] He worked with inoculated rabbits and studied their bone marrow during the course of the disease and postmortem. He explained the disappearance of the polymorphonuclear leukocytes from the marrow in any acute infection as due to a direct toxic action of the infection on the marrow, rather than to a simple emigration of the cells.

In another paper, Longcope[5] cites 2 cases of acute pneumonia in which the leukocyte count during life was below normal and the necropsies revealed an

[3] Jour. Path. and Bacteriol., 1901, 7, p. 161; Tr. Path. Soc., London, 1902, 53, p. 379.
[4] Bull. Ayer Clin. Lab. of Penn. Hosp., 1907, 4, p. 6.
[5] Bull. Ayer Clin. Lab. of Penn. Hosp., 1905, 2, p. 2.

only a few cellular patches. His explanation of these observations is the possibility that the functional activity of the marrow cells in each of these patients was exhausted.

Schurr and Löwy[6] report 2 cases of acute pneumonia that gave constant hyperleukocytic blood counts during life and yet showed aplastic, fatty marrow necropsy. These cases were used as part evidence for disproving the importance of the bone marrow as a leukocyte-former in acute infections.

Dickson,[7] in citing cases of pneumonia with leukopenic and aplastic bone marrow, ascribes the absence of leukoblastic reaction to the fact that before or during the onset of the pneumococcus infection the individual might have been weakened by alcoholic excess, syphilis, or other similar conditions. As another cause of imperfect reaction of the marrow, he describes "gelatinous degeneration" of the blood-forming tissue. This is a change in the bone marrow brought about by a progressive diminution of its blood-forming constituents along with an absorption of the fats of the marrow. This condition may be either acute or chronic.

The object of the present study was to determine, if possible, the explanation of the striking variation in the number of circulating leukocytes among cases of lobar pneumonia. We were interested particularly in finding out whether there existed a close parallelism between the leukocyte count and the degree of hyperplasia of the marrow, as has been very generally assumed. Our observations have been made on 17 cases of acute lobar pneumonia which came to necropsy. Only adults were included in the series, since in the earlier periods of life the bone marrow in the long bones is normally so active that degrees of hyperplasia cannot be easily recognized.

In every case bone marrow was obtained from the upper third of the femur. The method of procuring the specimen has been described elsewhere.[8] The tissue was fixed in Zenker's fluid, embedded in paraffin, and stained with hematoxylin and eosin. The presence or absence of hyperplasia was determined by a careful study of the microscopic sections. Aplastic marrows were classified as negative. The hyperplastic marrows were arbitrarily divided into 5 groups, designated in the table by the following signs: ±, +, + +, + + +, and + + + +. The criterion for + + + + hyperplasia was an extremely cellular marrow, replacing practically all of the adipose tissue with evidence of active cell multiplication.

A detailed study of the types of cells present in the marrow was not made, although in the routine examination of the sections it was noted, that in the majority of the cases showing hyperplasia, the predominating cell appeared to be an undifferentiated marrow cell with large round, vesicular nucleus and almost entirely fatty marrow. In 2 other similar cases there was red mottling of the marrow. In 1 case with a high leukocyte count, the marrow presented

[6] Ztschr. f. Clin. Med., 1900, 40, p. 412.
[7] The Bone Marrow, 1908.
[8] Lambert, R. A.: Proc. New York Path. Soc., 1918.

nongranular or finely granular cytoplasm. Polymorphonuclear leukocytes were present in the marrow of practically every case, and in several cases they were quite numerous. In this particular, our observations disagree with those of Muir and Longcope, to which reference was made in the previous paragraph.

The accompanying table shows the sex and age of the patients, the duration of the disease, the total and differential leukocyte count during life, and the state of the marrow at necropsy. An analysis of

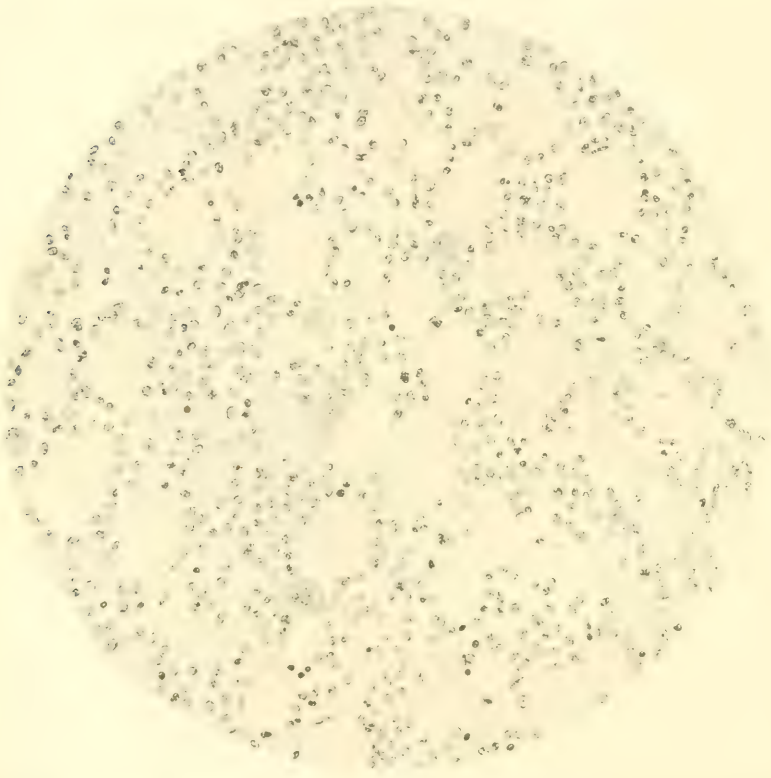

Fig. 1.—Markedly hyperplastic bone marrow from Case 17, in which the leukocyte count during life varied between 18,000 and 9,000, with a terminal count of 10,000 and 90% polymorphonuclears.

the data in individual cases gives varying results. In a few of the cases (Nos. 1, 4, 10 and 14) a close parallelism between the leukocyte count and the bone marrow changes is found. For example, in Case 4, in which the leukocyte count was persistently low, an aplastic marrow was found; and in Case 14, there was an association of high

leukocyte count and a markedly hyperplastic marrow. On the other hand, in a majority of the cases, no such agreement is seen. Indeed, several cases present a striking lack of harmony between the bone marrow and blood changes. For example, Case 17 (Fig. 1), in which there was a relatively low leukocyte count, a ++++ hyperplasia of the marrow was present. This, too, in a man of 42 years, while in Case 16, showing a moderately high leukocyte count, the bone marrow was found to be aplastic.

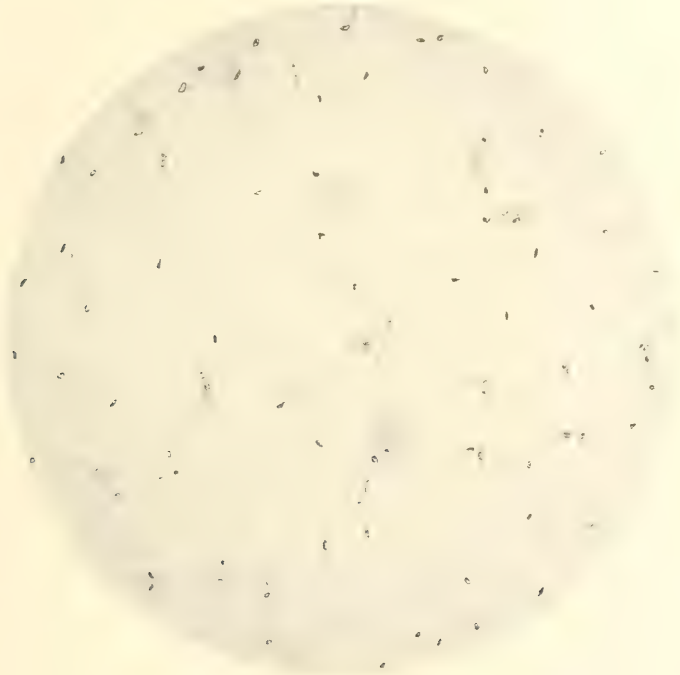

Fig. 2.—Aplastic bone marrow from Case 6, in which the leukocyte count during life varied between 17,000 and 21,000 with 90% polymorphonuclears.

An examination of the table shows that of the 17 cases, 11 exhibited varying degrees of hyperplasia of the marrow, and in 6 the marrow was aplastic. Of the aplastic cases, only one (Case 4) showed a persistently low leukocyte count. In another (Case 5), the count ranged from 28,000 to 41,000 during the earlier periods of the disease with a final count of only 4,000. In the other four cases, the counts averaged 20,000 with 90% polymorphonuclear leukocytes.

LEUKOCYTE COUNT AND BONE MARROW CHANGES IN LOBAR PNEUMONIA

Case	Sex	Age	Days Duration	Range		Final		Bone Marrow
				White Blood Cells	Polymorphonuclears	White Blood Cells	Polymorphonuclears	
1	M	39	30	17,000	95-65	14,000	94	+
2	M	55	17	16,000-	90-98	25,000	98	+ − +
3	M	71	16	48,000-2[illegible]	94-92	29,000	92	+
4	M	27	5	9,000-	88-74	7,000	74	−
5	M	43	21	28,000-4[illegible],000	88-92	4,000	83	−
6	M	29	9	17,000-21,000	89-90	21,000	90	−
7	M	48	6	4,000-30,000	84-92	30,000	87	−
8	F	58	12	42,000-42,000	88-88	42,000	88	+
9	F	50	2	28,000-43,000	87-93	43,000	93	+ −
10	M	29	5	17,000- 4,000	92-55	4,000	55	±
11	M	48	7	12,000- 4,000	94-60	4,000	60	+ −
12	M	22	4	9,000-15,000	95-90	15,000	90	+ +
13	M	55	28	20,000-13,000	98-81	15,000	88	−
14	F	32	2	47,000-47,000	94-94	47,000	94	+ + +
15	M	53	1	29,000-29,000	99-99	29,000	99	+
16	M	29	4	19,000-27,000	92-93	27,000	93	−
17	M	42	9	18,000- 9,000	96-88	10,000	90	+ + + +
Average	..	43	10	22,000-23,000	92-85	21,000	86	

SUMMARY

A close parallelism in the leukocyte count and the degree of hyperplasia of the marrow was found in less than half of 17 fatal cases of lobar pneumonia, which were studied. A few showed relatively inactive or aplastic marrows, with a leukocyte count well above normal. On the other hand, there were several cases in which the leukocyte count was persistently low during life, but in which a markedly hyperplastic marrow was found at necropsy.

To explain an aplastic femur marrow (a condition almost certainly common to all the long bones in these cases) associated with a leukocytosis during life, we may assume either a hyperplasia of the marrow of the flat bones only—a very improbable condition—or a formation of leukocytes outside the marrow, most probably in the spleen. The presence of large numbers of cells of the myeloid series in the splenic pulp would favor the latter explanation.

The cases of marked hyperplasia of the marrow with low leukocyte count are not easily interpreted. We could not find evidence in these cases of such rapid spread of the lesion as would account for a low leukocyte count through the draining of these cells out of the circulation.

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VALUE OF THE WASSERMANN REACTION.*

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NEW YORK.

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IN the twelve years which have elapsed since the method of complement fixation was applied by Wassermann to the diagnosis of syphilis, an enormous amount of clinical and serological data has been collected and published bearing upon the question of the specificity of the test. It was early recognized that the reaction was not, strictly speaking, a specific one, in that in carrying out the test it was not necessary to use as antigen either the syphilitic virus or any essential derivative of it. Nevertheless, reports from numerous observers soon brought general acceptance of the view that a positive reaction was obtained in a high percentage of cases of clinical syphilis, and that among non luetic cases a negative reaction was the rule.

While more recent reports have in general served to confirm or even broaden the earlier claims as to the value of the test, increasing emphasis has been placed by serologists on the delicacy of the re-

*A correlation of the seriological and post-mortem findings at the Presbyterian Hospital, New York.

action and the possibility of variations in results from relatively slight modifications in technique. It has been recognized, too, that certain conditions, such as acute infections associated with high fever, may give rise to positive reactions in non-luetic cases.

Very conservative, or even sceptical, views regarding the practical value of the test have not been lacking. Here and there reports have appeared in which it has been maintained that not only is the reaction negative in a large percentage of cases of syphilis, but also that positive reactions are so often reported in cases in which there is no clinical evidence of syphilis, that the test may be very misleading, if considered alone. An article embodying essentially this view, by Symmers and his co-workers at Bellevue Hospital, appeared recently in the *Jour. Amer. Med. Assn.*, 1918, LXX, 279. Since we shall have occasion later to refer to the results obtained by these authors, it may be well to quote their conclusions:

"1. Depending on the antigen employed, the Wassermann reaction in the living patient, as carried out at Bellevue Hospital, gives a negative result in from 31 to 56 per cent. of cases in which the characteristic anatomic signs are demonstrable at necropsy.

"2. The Wassermann reaction in the living patient is positive in at least 30 per cent. of cases in which it is not possible to demonstrate any of the anatomic lesions of syphilis at necropsy."

The Wassermann test is being widely used, and, as Symmers and his assistants point out, much reliance is being placed on it by Boards of Health, hospitals, practitioners, and the public. The question of the reliability of the test is therefore one

of very great importance. If the results obtained at Bellevue Hospital, which show so large an error in both directions—that is, in the high percentage of negative reaction in syphilis and in the demonstration of many positive reactions in non-syphilitic cases—have a general application, then the wide use of the Wassermann test is not only of doubtful benefit, but even a source of much harm, unless this wide margin of error be clearly recognized.

The importance of this question led us to make a comparative study of the serological and autopsy findings at the Presbyterian Hospital, similar to that made by Symmers and his coworkers at Bellevue Hospital.

The Wassermann test has been used in the Presbyterian Hospital some six or seven years, but it is only in the past three years that a uniform technique has been consistently followed, and of which the details can be presented. During the preceding years the serological work was done by several laboratory workers; and since we have no record of the details of the technique followed, except as to the antigen employed, we have thought it advisable to present and discuss only the data compiled in this last three-year period.

The autopsies during this period were performed by or under the immediate direction of Professor W. G. MacCallum, Dr. A. M. Pappenheimer, and Dr. R. A. Lambert of the College of Physicians and Surgeons. Complete autopsy protocols were recorded in every case, including gross and miscroscopic description of all organs—with the exception of the central nervous system in a certain percentage of the cases. In every instance, microscopic sections were preserved, and have been studied again in the preparation of this report. It has been

possible, also, to reexamine many of the gross specimens, which had been preserved in the museum for teaching purposes. In this review of the pathological material, however, a change in diagnosis was made in only three instances. Two cases showing microscopically definite syphilitic aortitis had been overlooked and the syphilitic nature of a scarred liver had, in one instance, not been recognized. It should be clearly stated that only those cases which showed definite syphilitic lesions were placed in the group of positive anatomical syphilis. Those showing suspicious, but not clear cut luetic lesions—a relatively small number of cases—were placed in the doubtful group.

Since differences in results obtained with the Wassermann tests are undoubtedly due in large part to differences in technique, we shall give in some detail the method in use at the Presbyterian Hospital.

Two antigens are used in all tests: a crude alcoholic extract of guinea pig heart and a cholesterinized extract of guinea pig heart. The ice-box fixation method for four hours is employed with the alcoholic antigen; water bath fixation at 37 deg. C. for ½ hour with the cholesterinized antigen. Serum is inactivated ½ hour at 55 deg. C. The sheep system is used. Complement for each guinea pig is titrated separately, against 1½ units of amboceptor, and is so diluted that a dose of 1½ units is used in the alcoholic tests, a dose of two units in the cholesterin tests. Each specimen of complement is also tested with varying amounts of the cholesterin antigen, the amount to be used in the tests, twice, three times, and four times that amount. Any specimen that fails to hemolize completely in the presence of the cholesterin antigen

is not used in the cholesterin tests, but it may be used with the alcoholic antigen, if its hemolizing power is good, according to the complement titration. An amboceptor titration is made with each mixture of complement; that for the alcoholic tests and that for the cholesterin, and for sensitizing the cells, the amboceptor is so diluted that one dose contains $2\frac{1}{2}$ units. Tests are read as soon as hemolysis is complete in the antigen and serum controls, in which there is double the amount of antigen and serum used in the tests. By a ++++ reaction is meant complete inhibition of hemolysis in 0.2 c.c. of serum. Degrees of incomplete inhibition are indicated roughly by +++, ++ and +. Only a ++++ reaction is considered positive.

TABLE I.

Autopsy Findings.	No. Cases.	Wassermann	Wassermann	Wassermann	
		++++ Both antigens	0 Both antigens	+, ++, or +++ with either antigen	
Positive	23	18	3	2 (trtd.)	85.7 per cent positive (excluding 2 treated cases giving doubtful reactions).
Negative	188	0	175	13	
Doubtful	3	2	0	1	
Total.....	214	20	178	16	

The results of the comparison of the serological and autopsy findings can be best presented in the form of tables. On the basis of the post-mortem findings, the cases are divided into three groups: (1) syphilitic or "positive" cases, (2) non-syphilitic or "negative" cases, and (3) doubtful cases. Table

I shows at a glance the results of the Wassermann test in these three groups.

It is seen that of 23 cases showing definite syphilitic lesions, 18 gave + + + + Wassermanns (both antigens), 2 (treated cases) gave doubtful reactions with one or both antigens, and 3 gave negative reactions. That is, in 18 out of 21, or 85.7 per cent. of cases of proven, *untreated* syphilis, a + + + + Wassermann was obtained during life. It will be recognized that this percentage of positive Wassermanns is relatively high, when consideration is taken of the fact that most of the cases were examples of late syphilis, as indicated in Table IV. There were three cases in which the autopsy findings were not conclusive, and which are classed as doubtful syphilis. One was an example of a coarsely nodular cirrhosis of the liver associated with a chronic interstitial orchitis and chronic meningitis of possible leutic nature. The second was a case of nodular cirrhosis of the liver with an inflammatory reaction which suggested lues. In the third case, death was due to lobar pneumonia, but chronic interstitial orchitis was found, which suggested a possible old, leutic infection. The last two cases gave a + + + + Wassermann reaction, the first a doubtful reaction.

The most striking fact, in our opinion, brought out in the table, is, that of 188 *cases showing no evidence whatever of syphilis at autopsy, not one had given a + + + + Wassermann during life.*

In Table II the data are so arranged as to bring out the significance of the Wassermann in the light of post mortem findings. In other words, the figures constitute the pathologist's answer to the question as to what a positive, negative or doubtful Wassermann reaction means. The table shows that

20 cases gave a ++++ Wassermann with both antigens, and that, in 18 of these, definite evidence of syphilis was found at autopsy. One of the two remaining cases showed suspicious lesions in the liver, very probably syphilitic, and the other a chronic orchitis, possibly syphilitic, but since this second case was one of pneumonia and was bled while running a high temperature, the ++++ Wassermann may be regarded as non-specific. It is seen, therefore, that 18 out of 20, or 90 per cent. of positive Wassermann reactions were conclusively confirmed by autopsy findings, and that in the two remaining cases suspiciously syphilitic lesions were present—a possible margin of error of 10 per cent. Of 178 patients giving negative Wassermann reactions with both antigens, only 3 showed definite syphilitic lesions at autopsy. Hence, the negative Wassermanns were confirmed at autopsy in approximately 98 per cent. of the cases, a margin of error less than 2 per cent.

TABLE II.

Wassermann.	No. Cases.	Autopsy Findings		
		Pos.	Neg.	Doubtful.
++++ (both antigens)	20	18	0	2
0 (both antigens)	178	3	175	0
+, ++, +++ (with either antigen)	16	2*	13	1
Total	214	23	188	3

*Treated cases.

Sixteen patients had given a doubtful (+, ++, or +++) reaction with one or both antigens. Two were cases of definite syphilitic aortitis which were

being treated. One case showed suspicious lesions in the meninges, liver, and testes at autopsy. In the 13 remaining cases, no anatomical evidence of syphilis was found. Excluding the two doubtful reactions in the cases under treatment, 13 out of 14, or 93 per cent., approximately, of the doubtful Wassermann reactions were non-specific as judged by autopsy findings. It is a fact worthy of note, that 11 out of 16, or 69 per cent., of these doubtful reactions were given by the cholesterin antigen only. Four specimens gave a doubtful reaction with both antigens,—one a treated case of syphilis, and one a case of possible syphilis. In only one instance (a case of sarcoma of the ovary) was there a doubtful reaction with the alcoholic antigen alone. It is thus evident, that while the cholesterin antigen may have the advantage of giving more reactions in cases of syphilis, and of more nearly excluding syphilis, it has the disadvantage of giving a considerably larger number of apparently non-specific reactions, as judged by autopsy findings.

Discussion.—The data presented above show a very close parallel between the serological and autopsy findings at the Presbyterian Hospital during the past three years. Though the number of cases is somewhat smaller, the character of the material is very much the same as that which forms the basis of the report from Bellevue Hospital by Symmers and others quoted above, in which very different conclusions were reached. At Bellevue Hospital, a positive Wassermann was obtained in only 44 to 69 per cent. of the cases of syphilis demonstrated at autopsy, as compared with 85.7 per cent. at the Presbyterian Hospital. Furthermore, the authors report positive reactions in 30 per cent. or more of the non-syphilitic cases, whereas we

failed to find among such cases a single ++++ Wassermann, and only 7 per cent of doubtful (+, ++, +++) reactions.

TABLE III.—DOUBTFUL REACTIONS.

No. Cases.	Antigen.	
	Alcoholic.	Cholesterinized.
11	0	?
1	?	0
4	?	?
Total.. 16	? = +, ++, or +++	

TABLE IV.—CASES SHOWING POSITIVE AUTOPSY FINDINGS.

		Wassermann.		
	No. Cases.	++++	0	? (+, ++, +++, Either Antigen).
Syphilis of aorta and heart...	15	11	2	2 (treated cases).
Tabes	1		1	
Congenital syphilis	2	2	..	
Miscellaneous	5	5	.	
Total	23	18	3	2

The question naturally arises as to the reason for the difference in results at the two institutions.

The nature of the data, both pathological and serological, which forms the basis of reports of this kind, is such that a fair critical review is not possible except upon a close examination of the material itself, with careful regard to the details of the methods used.

We shall therefore make no attempt to analyze

the factors responsible for the differences between our results and those quoted, except for brief reference to one point:

At Bellevue Hospital the cholesterin-fortified antigen was used alone in a majority of the cases. In 49 listed cases, in which both alcoholic and cholesterin antigens were used, the results with the two antigens differed in approximately 60 per cent. In our series of cases at the Presbyterian Hospital the two antigens were used in every test with only 6 per cent. of discordant results.

Conclusions.—A comparison of the serological and autopsy findings at the Presbyterian Hospital during the past three years brings out the following facts:

(1) Among cases showing anatomical evidence of syphilis at autopsy 85.7 per cent. had given during life a ++++ Wassermann with both alcoholic and cholesterinized antigens.

(2) Among 188 cases showing at autopsy no anatomical evidence of syphilis, 93 per cent. had given during life a negative Wassermann with both antigens and 7 per cent. doubtful reactions (+, ++ or +++ with either antigen).

These findings, in our opinion, warrant the conclusion that the Wassermann test, properly carried out, can be relied upon as an important aid in the diagnosis of syphilis.

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THE VALUE OF THE WASSERMANN REACTION: A CORRELATION OF THE SEROLOGICAL AND POST-MORTEM FINDINGS AT THE PRESBYTERIAN HOSPITAL, NEW YORK

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The Wassermann reaction as a test for syphilis has been in general use for more than ten years, but there is still considerable difference of opinion, especially among clinicians, as to its diagnostic value. It is generally agreed that a majority of syphilitics, especially in the early cases, give a positive test. But there is a lack of agreement as regards, first, the percentage of cases of syphilis giving a negative reaction, and, secondly, the frequency of positive reactions in non-luetic cases. It is obvious that the diagnostic value of the test may be accurately measured by the answers to the questions implied in the two points just stated.

It is hardly necessary to mention the fact that numerous reports bearing upon the value of the test have been published in recent years. In most of the reports, however, the serological findings have been controlled only by the clinical history and physical examination of the cases, in which there is a well recognized wide margin of error. There can be but little question that the only reasonably accurate method of checking up the Wassermann test lies in a complete gross and microscopic examination of the body after death.

This method of estimating the value of the Wassermann reaction has been applied at Bellevue Hospital by Symmers and his co-workers, who have recently published a report, the conclusions of which are as follows:

"1. Depending on the antigen employed, the Wassermann reaction in the living patient, as carried out at Bellevue Hospital, gives a negative result in from 31 to 56 per cent. of cases in which the characteristic anatomic signs of syphilis are demonstrable at necropsy.

"2. The Wassermann reaction in the living patient is positive in at least 30 per cent. of cases in which it is not possible to demonstrate any of the anatomic lesions of syphilis at necropsy."

Since these results, if substantiated, would make questionable the wisdom of the present general use of the Wassermann test, it seemed to us important that more data bearing on the question should be collected. In pursuance of this object we have attempted a correlation of the serological and autopsy findings in cases coming to autopsy at the Presbyterian Hospital during the past three years. During this period a uniform method of carrying out the Wassermann test has been followed, and complete records of all autopsies have been kept, including gross and microscopic descriptions of every organ.

As the Wassermann technique has been subjected to various modifications, it may be well to give in some detail the essentials of the method in use at the Presbyterian Hospital.

TECHNIQUE

Two antigens are used in all tests: a crude alcoholic extract of guinea-pig heart, and a cholesterinized extract of guinea-pig heart. The ice-box fixation method for four hours is employed with the alcoholic antigen; water bath fixation at 37° for one half hour with the cholesterinized antigen. Serum is inactivated one half hour at 55° C. The sheep system is used. Complement from each guinea-pig is titrated separately, against one and one half units of amboceptor, and is so diluted that a dose of one and one half units is used in the alcoholic tests, and a dose of two units in the cholesterin tests. Each specimen of complement is also tested with varying amounts of the cholesterin antigen, the amount to be used in the tests, twice, three times, and four times that amount. Any specimen that fails to hemolyze completely in the presence of the cholesterin antigen is not used in the cholesterin tests, but it may be used with the alcoholic antigen, if its hemolyzing power is good, according to the complement titration. An amboceptor titration is made with each mixture of complement, that for the alcoholic tests and that for the choles-

terin; and for sensitizing the cells, the amboceptor is so diluted that one dose contains two and one half units. Tests are read as soon as hemolysis is complete in the antigen and serum controls, in which there is double the amount of antigen and serum used in the tests. By a ++++ reaction is meant complete inhibition of hemolysis in 0.2 c.c. of serum. Degrees of incomplete inhibition are indicated roughly by +++, ++, and +. Only a ++++ is considered positive.

The results of the serological and autopsy findings in the 214 cases analyzed can be best presented in the form of tables. On the basis of the post-mortem findings the cases are divided into three groups: (1) Syphilitic, or "positive" cases; (2) non-syphilitic, or "negative" cases, and (3) "doubtful" cases.

TABLE I

Autopsy Findings	No. Cases	Wassermann			
		++++ (Both Antigens)	(Both Antigens)	(With Either Antigen)	
Positive	23	18	3	2	85.7% Positive (Excluding 2 Treated Cases Giving Doubtful Reactions)
Negative	188	0	175	13	
Doubtful	3	2	0	1	
Total	214	20	178	16	

TABLE II

Cases Showing Positive Autopsy Findings

	No. Cases	Wassermann		
		++++	0	+, ++, or +++ (With Either Antigen)
Syphilis of aorta and heart	15	11	2	2 (treated cases)
Tabes	1		1	
Congenital syphilis	2	2		
Miscellaneous	5	5		
Total	23	18	3	2

Table I is designed to show at a glance the results of the Wassermann test in these three groups. It is seen that 18 out of 21 cases of proved untreated syphilis had given during life a

— — + + Wassermann with both antigens. A majority of these cases were examples of late syphilis, as indicated in Table IV. The most significant fact brought out in this analysis is that of 188 cases showing no evidence whatever of syphilis at autopsy, not one had given a + + + + reaction. Three cases were doubtful anatomically. In two of these, with very suspicious lesions in liver and testis, there was a + + + + Wassermann. In the third, a chronic orchitis, the reaction was negative.

TABLE III

Wassermann	No. Cases	Autopsy Findings		
		Positive	Negative	Doubtful
+ + — — (Both antigens)	20	18	0	2
0 (Both antigens)	178	3	175	0
+, —+, + — (With either antigen)	16	2	13	1
Total	214	23	188	3

In Table III the data are so arranged as to bring out the significance of the Wassermann reaction in the light of post-mortem findings. In other words, the figures constitute the pathologists' answer to the question as to what a positive, negative, or doubtful Wassermann reaction means. The table shows that 20 cases gave a + + + + Wassermann test with both antigens and that in 18 of these characteristic syphilitic lesions were found at autopsy. One of the two remaining cases showed suspicious lesions in the liver, very probably luetic in origin, while in the other there was a chronic orchitis, possibly syphilitic. It is thus seen that 18 out of 20, or 90 per cent. of the positive reactions, were conclusively confirmed by autopsy findings and that in the two remaining cases there were lesions strongly suggestive of syphilis, a possible margin of 10 per cent. Of 178 patients giving negative reactions with both antigens, only three showed definite syphilitic lesions at autopsy. That is, the negative Wassermanns were confirmed in approximately 98 per cent. of the cases—a margin of error of only 2 per cent.

There were 16 cases giving reactions classified as doubtful (+,

++, +++) with one or both antigens. Two of these were cases of treated syphilis; one showed lesions in meninges, testis, and liver, possibly syphilitic in origin. In the 13 remaining cases no anatomical evidence of syphilis was found. Excluding the two doubtful reactions in treated cases, 13 out of 14, or 92.8 per cent., of the doubtful Wassermanns were non-specific as judged by autopsy findings. It is a noteworthy and possibly significant fact, that 11 out of 16, or 68.7 per cent., of these doubtful reactions were given by the cholesterin antigen only. In only one instance was there a doubtful reaction with the alcoholic antigen alone. It is thus evident that while the cholesterin antigen may have the advantage of giving more reactions in cases of syphilis, and of more nearly excluding syphilis, it has the disadvantage of giving a considerably larger number of apparently non-specific reactions, as judged by autopsy findings.

TABLE IV

Doubtful Reactions

No. Cases	Antigen	
	Alc.	Chol.
11	0	?
1	?	0
4	?	?

Total 16 ? = +, ++, or +++

CONCLUSIONS

A comparison of the serological and autopsy findings at the Presbyterian Hospital during the past three years brings out the following facts:

1. Among cases showing anatomical evidence of syphilis at autopsy 85.7 per cent. had given during life a ++++ Wassermann with both alcoholic and cholesterinized antigens.

2. Among 188 cases showing at autopsy no anatomical evidence of syphilis, 93 per cent. had given during life a negative Wassermann with both antigens and 7 per cent. doubtful reactions (+, ++, −++ with either antigen).

These findings, in our opinion, warrant the conclusion that the Wassermann test, properly carried out, should be of great aid in the diagnosis of syphilis.

Discussion:

Dr. Norris: I do not think that I care to discuss this matter. I distinctly recognize the value of these papers, and they are quite in keeping with a number of papers published before. The subject does not lend itself easily to discussion and since there has been more or less of an individual attack in the first paper I do not think it would seem suitable on my part to make a reply to a reply. There are a certain number of unwarranted assumptions in the paper of Dr. Larkin. In the first place he seems to assume that Dr. Symmers did all the serological tests himself. He did not do them at all. They were made either by Dr. Field or Dr. St. George, or under their direction. The methods that were used are known as Dr. Field's; certainly his publications are of some value, and Dr. Symmers' attempt was merely to correlate the gross findings with the results obtained in general laboratory routine work. I do not care to uphold the dignity of Bellevue, because I do not think it necessary. About three or four years ago in doing routine pathological autopsy work I came across a number of cases in which there were fifteen or eighteen with positive Wassermanns, and the first two of them opened my eyes thoroughly. At that time I think Dr. Field was doing only the cholesterin test. So far as we could tell at autopsy there was no evidence of specific infection; there had been no treatment, and there was no history of syphilis. When you run across a certain number of such cases they disconcert you. However, I see no reason for further discussion. Dr. Symmers is amply and fully able to take care of himself.

Dr. Ottenberg: I also do not feel very much like entering into a discussion, as apparently there is something of the personal element involved. I will say this, that until Dr. Norris spoke I felt that there was nothing further

to say because it seemed to me that the two papers which had been read settled the subject most conclusively. I still feel that, too. When Dr. Symmers' paper appeared, like everyone else who was familiar with the subject, I thought that if he was right, I must have been deceiving myself very greatly in over 20,000 Wassermanns I have personally done in which I have investigated the clinical histories and wherever I have been able to, have followed the case to autopsy. As a matter of fact, after reading the paper more closely, it answers itself. I have no personal bias in the matter at all, but in going over the figures, the number of inconsistencies between the tests with simple alcoholic and those with cholesterin reinforced antigens was so enormous that one or the other methods must be at fault. Now I know that in the use of the cholesterin antigen Dr. Field's method had been followed at Bellevue. In testing out methods used in various institutions in New York City a few years ago I tried out that method. and the records showed a larger percentage of errors with Dr. Field's method than with any other. However, I did not conclude that the method was to be rejected at that time, but held judgment in reserve, because I had only used it in some two hundred cases. Later I gave it up in order to try other methods, and finally used the Walker and Swift one as the most reliable with the cholesterin antigen. I finally recognized, as Miss Olmstead says, that there is a certain number of non-specific results with cholesterinated antigen, but it seems to me that with this knowledge, and with the knowledge of the source of these errors on one side or another, it is possible to explain what has occurred.

Dr. Ewing: I agree with Dr. Norris that the subject of the Wassermann reaction does not lend itself well to discussion. There are so many intricate questions involved on both sides that the matter can hardly be settled by any form of debate. If there is any personal bias in the papers presented, this is doubtless only a sign of the healthy interest that is quite generally taken in the merits and demerits of the Wassermann reaction. I also think that Dr. Symmers' contribution will take care of itself and I have no intention of attempting its defense.

It seems to me, however, that it is very difficult to make any significant comparison of the results of the Wassermann reaction and the anatomical signs of syphilis in the body, because there are so many variable factors in both fields. Consider, for instance, the uncertainties regarding the technique of the Wassermann reaction. Not long ago several laboratories in New York consulted together and endeavored to establish a standard technique. The results were so divergent that the conclusions were never published, and there has been continuous recrimination between technicians regarding the different methods adopted. Anyone listening to Miss Olmstead's exhaustive presentation will realize how complex and delicate a thing this Wassermann technique is.

On the other side it must be recognized that the subject of the anatomical signs of syphilis is a very large one. There is hardly anything that requires more experience on the part of the pathologist than the identification of the lesser lesions of syphilis by gross and microscopical tissue changes. I think it quite likely that any two pathologists might differ regarding certain pos-

sibly syphilitic lesions, unless the *Spirochæta pallida* were demonstrated in the lesion. In the demonstration of spirochetæ we have the only positive sign of syphilis, but these organisms are often very difficult to find and may be absent in old syphilitic scars. Nevertheless, the organs of subjects who had had well marked syphilis during life regularly show rather definite lesions, the character and significance of which have been established by the older pathologists by much labor extending over many years.

The great weakness of the Wassermann reaction is that it is not a specific reaction. What possible essential relation can there be between an alcoholic extract of heart muscle of a guinea-pig and the bodies of *Spirochæta pallida?* I do not think that serologists have paid enough attention to the exact meaning of this reaction, which seems to indicate only the presence in the blood of an antigen reacting against derivatives of red blood-cells or other chemically identical substances. Based on this non-specific test, clinical medicine, chiefly in the United States, has gone to great lengths, and even committed some absurd blunders. One of our enterprising Western States decided that it was necessary to have a negative Wassermann reaction before anyone could have a marriage license, and they passed a law to that effect. But the law was soon pronounced unconstitutional by their Supreme Court; it was found that the laboratories of the State could not carry out the work, and it is also clear that the law is medically unsound. In several diseases, notably in chronic gastritis, clinicians have attempted to establish a syphilitic etiology on the basis of the Wassermann reaction. Now there is no doubt that this reaction is of value in enabling clinicians to take care of the sick, but when it comes to establishing new syphilitic diseases by means of such evidence, it is time to protest. Even as a clinical test it has seemed to me to have very definite limitations which are seldom emphasized by its sponsors, and I have often been deeply impressed by the errors into which experienced clinicians have been led by too exclusive reliance on the test. Moreover, it is just in these doubtful cases which have appeared in one of the papers this evening, that one wants help from the Wassermann; and it is just in such cases that the Wassermann fails.

There is quite a long list of diseases in which the Wassermann reaction occurs frequently, including malaria and other acute infections, tuberculosis, leprosy, jaundice, pernicious anemia, hemoglobinemia, and even acidosis.

I am inclined to think that the limitations of the Wassermann reaction are being recognized more and more by conservative clinicians and laboratory men, and I know of several who speak with much less confidence about the test now than they did some years ago.

Dr. Moschcowitz: I wish to make a few remarks from the viewpoint of the clinician. I believe a distinction should be made between interpreting the value of the Wassermann reaction from the standpoint of the pathologist and from the standpoint of clinical findings. Certainly in the wards of a hospital and in general practice the Wassermann reaction bears out clinical expectations very closely. In other words, a positive Wassermann reaction is only exceptionally found when it has not been expected; the converse is equally true.

Dr. Larkin: As one of the contributors to this paper and as a member of the Pathological Society I wish to say that in the presentation of this subject no personal bias has tinctured our remarks or influenced our conclusions. Now I think it is the attitude of our scientific men, and it has been the attitude of the Pathological Society, to have an impersonal and unbiased discussion of any scientific subject, and I feel that my colleague, Dr. Norris, has taken an extremely unjust attitude, which I highly resent, in his remarks on our presentation. We have all met here many a time in a "healthy" way for discussion, and our ideas should be exchanged without indulging in any personalities. In preparing this paper it has been the ambition of the authors to eliminate the personal side. I cannot discuss at length the figures which have been gone over because there are other papers to be presented here. I only wish to say that one or two of Dr. Ewing's remarks might bear some discussion. One of them is his observation on the Wassermann reaction in tuberculosis. It has been my endeavor to study personally the character of the reaction in pretty nearly 20,000 cases of tuberculosis at an early stage which have been very carefully gone over. This is a task requiring much labor, and perhaps when we have more knowledge of the Wassermann reaction in syphilis we may be able to present some "healthy" discussion on the subject. Of course, Dr. Ewing is something of an iconoclast, and it is hard to convince him. One remark is noteworthy, and that is the attitude of the surgeon in regard to syphilis. From a very large experience I have seen only two cases of gastric syphilis, though I have seen a number of cases which have been sent to me, but which showed no evidence of the lesion in the gross or microscopically, despite the fact that the clinician and the surgeon very likely made the assertion that the individuals had syphilis.

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CASES OF TYPICAL AND ATYPICAL LYMPHOSARCOMA

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The classical descriptions of Kundrat (1) and Paltauf (2) differentiated lymphosarcoma from the group which in the older literature had all been called malignant lymphomas. The conception of Sternberg, who reviewed the subject in 1903 and added his own observations, is briefly as follows (3): Lymphosarcoma is a tumor formation starting in a group of lymph nodes and spreading thence to neighboring nodes or follicles. It spreads from region to region without ever exhibiting such general lymphoid involvement as leukemia or pseudoleukemia. Sooner or later it invades the capsule and extends into the surrounding tissue. Metastases differ from those of ordinary neoplasms in that the intervening lymphoid tissues are affected. True metastases, which can be explained only through transfer by way of the blood stream, are rare, and usually isolated. Blood-vessels are seldom invaded by lymphosarcoma, being usually only surrounded and narrowed. The commonest sites of origin are the lymph nodes of the neck, mediastinum, mesenteric and retroperitoneal regions; less frequently the inguinal and axillary nodes. The affected groups form nodular, uneven masses, well limited in the beginning, but later diffusely permeating the surroundings; they are mostly hard, grayish-white, and show a homogeneous cut surface. In general, when a hollow organ is invaded, the growth tends to form a sleeve-like mass about it, usually, however, causing no obstruction, but, on the contrary, tending to widen the lumen. The spleen is rarely attacked and the bone-marrow likewise. Histologically, the tumor is characterized by an irregular reticular framework with lymphoid

cells in the meshes; the architecture of the node is lost; follicles and medulla are no longer differentiated, and the capsule and surrounding tissue are diffusely infiltrated with tumor cells so that the separate lymph nodes can neither be distinguished from one another nor from the surrounding tissue. Often the tumors show an alveolar structure. The cells resemble lymphocytes but are larger, have a more lightly staining nucleus, and a scanty, often almost invisible, non-granular cytoplasm. There is no definite alteration of the blood picture.

The eight cases described by Dr. MacCallum (4) fall quite clearly into this group. Of his cases, none of the three which belonged to the intrathoracic type showed metastases in distant organs. The dissemination of the tumor masses conformed strictly to Kundrat's idea of regional distribution. Indeed, in one of these cases, the tumor had penetrated the heart wall and hung in polypoid masses within the heart without metastasizing by the blood stream. Of the five cases in which the intestinal lesion forms the constant feature, two showed single metastatic nodules in the liver and another had metastases in the liver, thymus, bone-marrow, and kidneys; but these, from the description, seem to have been more like infiltrations, using the framework of the organ as their support, rather than metastatic nodules. The structure of the tumors and the characteristics of the cells, as well as the pathological anatomy in Dr. MacCallum's cases, agree quite closely with Sternberg's description of the Kundrat-Paltauf lymphosarcoma.

The descriptions by Kaufmann (5) and Naegeli (6) agree with those mentioned. The former, however, speaks of two types of lymphosarcoma: the regional and the generalized. The latter he regards as rare, but reports a case of a man, twenty-five years of age, in which a primary lymphosarcoma of the small intestine was associated with metastases in the skin, pericardium, pleura, lung, bone-marrow, and kidneys. Naegeli, too, observes that in the late stages one may find widely distributed nodules.

It is clear, then, that the occasional occurrence of a widespread dissemination of metastases has been well recognized, but

in general these colonies have been described as diffuse infiltrations rather than as distinct circumscribed nodules. The spleen has been considered as almost immune to the tumor.

The four cases included in this report have been taken from the autopsy records of the past five years at the Presbyterian. It would take too long to attempt to give a complete account of each case, but the summaries include the essential features.

Case I. A man of fifty-eight, who six months before admission to the Presbyterian Hospital, in September, 1912, noticed a large lymph node in the left groin. Three weeks later the superficial lymph nodes all over the body were enlarged. The legs, and then the abdomen and scrotum, became swollen. Physical examination showed great enlargement of all the superficial lymph nodes, fluid in the abdomen, enlargement of the liver, and oedema of the external genitals and lower extremities.

Blood count.

Red blood cells	3,600,000
Haemoglobin	80 per cent
White blood cells	9,600
Polymorphonuclears	79
Lymphocytes	16
Large mononuclears	5
Eosinophiles	0

Three weeks later

White blood cells	12,000
Polymorphonuclears	80 per cent
Small lymphocytes	12 per cent
Large lymphocytes	6 per cent
Eosinophiles	3 per cent

All the lymphatics steadily enlarged and fluid accumulated in the chest. The spleen enlarged so that the edge was felt 6 cm. below the costal border; the edge of the liver was 7.5 cm. below the costal border. A large, firm mass was felt in the pelvis on rectal examination. There is no note in the record of an examination for Bence-Jones protein.

Autopsy. There was a great enlargement of all the superficial lymph nodes. Surrounding the rectum, and nearly filling the pelvis, to the walls of which it was adherent, there was a tumor which extended upward and involved the retroperitoneal lymph nodes, surrounding each ureter, the aorta, and the inferior vena cava. Extend-

ing from the mass about the inferior cava, the tumor had infiltrated the right kidney, diffusing itself gradually out to the cortex. The pericardium was studded with irregular nodules and was adherent to both lungs. The pleural surface of each lung was marked with small nodules and tumor tissue was also found passing into the lung about the bronchi. The mediastinal and bronchial nodes were much enlarged. The liver contained no tumor nodules. The spleen measured 18 by 10 by 7 cm. It showed some small anaemic infarcts and on the cut surface innumerable small white foci appearing like enlarged malpighian corpuscles. There was no tumor in the pancreas nor in the left kidney. The oesophagus had a great many very small nodules on the mucous membrane. The stomach and intestine exhibited no evidence of tumor, nor was the lymphoid tissue unusually prominent. The bone-marrow of the femur contained some very small nodules scattered through it.

Microscopical examination. Microscopically the tumor has the same appearance wherever found. The architecture of the lymphoid tissue is entirely lost, the tissue being overwhelmed by tumor cells. The stroma is scanty; the tissue is only moderately vascular. The cells show considerable uniformity in size and shape; they are a little larger than lymphocytes, stain less deeply, and have little or no visible cytoplasm. No tendency toward an alveolar structure is evident. The capsule of the lymph nodes has been invaded, and dense accumulations of cells are found scattered diffusely through the surrounding tissue. In the spleen, the tumor cells have formed follicle-like masses two or three times the usual size of malpighian corpuscles.

Case II. The patient was a man of fifty-five admitted to The Presbyterian Hospital January 4, 1916. He had had a chancre and secondary lesions twenty-three years ago. For seven months before admission to the hospital he had had intermittent sharp darting pains in the back, about the shoulders, and in the groins. Various forms of treatment gave no relief. Physical examination showed a stout plethoric man with slight cyanosis, a little exophthalmos, nystagmus and inequality of the pupils. Heart a little enlarged to the left, systolic murmur at the apex, and occasional extra systoles. There was also a right inguinal hernia. Superficial lymph nodes were not enlarged. Neither liver nor spleen was palpable.

Blood count.

	JANUARY 3	JANUARY 7	JANUARY 8	JANUARY 10	FEBRUARY 1	FEBRUARY 10
White blood cells.....	18,000	25,000	16,800	20,600	14,400	18,200
Polymorphonuclears....	70	73	78	73	70	73
Small lymphocytes.....	30	22	17	21	23	11
Large lymphocytes.....	0		2	5	2	
Eosinophiles.....	0	2	0	1	0	2
Transitionals.....	0	3	2	0		
Haemoglobin.....	90 per cent				5	14

Wassermann. Alcoholic antigen, negative; cholesterin antigen, negative.

Spinal fluid. Normal.

Phthalein. 28 per cent in two hours.

McLean Ambard index. 95.5.

Examination of stool. No blood, pus, ova, or parasites.

Gonococcus complement fixation. Negative.

Pain in various parts of the body persisted, he became paralyzed below the waist, lost control of bladder and rectum, and developed oedema of the legs. By rectal examination a mass was felt above the prostate and hard irregular masses were noted in the left side of the abdomen.

Urine. Specific gravity, 1020 to 1037. Acid. Albumin—very faint trace. Glucose, none. Microscopic, hyaline and granular casts.

Temperature and pulse. Normal till just before death.

Blood pressure. 124/74.

Autopsy. The lymph nodes along the aorta and in the mesentery were greatly enlarged. The grayish tissue by which they were replaced had extended through their capsule and invaded the fat about them, forming a large mass almost completely surrounding the aorta and vena cava from the diaphragm to the pelvis. There were nodules also in the fat about both kidneys. The bronchial lymph nodes were enlarged and composed of tumor tissue. A single node in the lower part of the cervical chain, measuring 1 cm. in diameter, was composed of tumor. The pleural surface of each lung contained nodules, and numerous whitish tumors projected from the liver, the largest measuring 2 cm. in diameter. The spleen weighed 85 grams and seemed to be normal. The dura of the spinal cord, from the 8th dorsal vertebra upward, was infiltrated by a sheet of tumor measuring 1 to 3 mm. in thickness. It had surrounded the roots of the nerves as they passed

out, but did not appear to have exerted pressure on the cord itself, nor were the vertebrae invaded. The other organs showed nothing of interest.

Microscopical examination. Here again the invasive quality and loss of the lymphoid tissue are evident in the sections. In vascularity and in the amount of connective tissue framework, this case is quite similar to the first, but in both the primary tumor and the metastases there is a more marked tendency toward an alveolar arrangement.

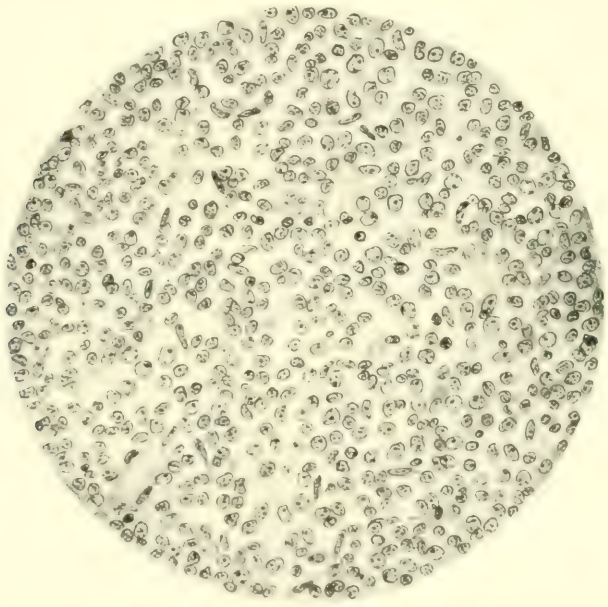

FIG. 1. CASE II

Drawing showing character of tumor cells in the metastatic nodules in pleura and liver. The cells are slightly larger than lymphocytes, are more irregular in shape, and have vesicular nuclei

Except for their slightly greater size, the cells of the primary growth in the abdominal lymph nodes resemble normal lymphocytes quite closely; they are 8 to 10 micra in diameter, the nucleus is round, deeply stained, and surrounded by only a narrow rim of cytoplasm. In the liver, spinal meninges, and pleural metastases, the cells are larger, more irregular in shape, and have more vesicular nuclei. In the bone marrow there are small discrete foci of tumor cells.

These two cases, it seems, may be classed as belonging to the Kundrat-Paltauf group. In the first, the primary tumor was apparently about the rectum: from here it had extended to practically all the lymphoid tissue in the body and had extensively infiltrated the surrounding tissues. The foci in the spleen and marrow are unusual features but the occasional occurrence of such metastases has been recognized. The primary growth in the second case was evidently in the lymph nodes about the aorta. The invasion of the spinal meninges with the clinical picture dominated by spinal cord symptoms, and the discrete nodules in the liver, were the atypical features here.

Microscopically, both of these cases agree well with the descriptions of Drs. MacCallum, Sternberg, Paltauf, and Kundrat.

The two following cases are more atypical, and hence more difficult to classify.

Case III. The patient was a Russian woman of forty-four, who entered the Presbyterian Hospital August 1, 1916. There was nothing important in the family history or past history.

Seven months before admission to the hospital she noticed enlarged nodes in each inguinal region. Three months later, those in the left axilla became enlarged and subsequently all the other superficial lymph nodes. On physical examination there was found general enlargement of all the superficial nodes, without any tenderness; signs of fluid in the left chest; an enlarged liver, the anterior margin of which extended 8 cm. below the costal border; in the left upper quadrant of the abdomen a very large mass with a notched border taken to be the spleen, and nodular masses in the left lower quadrant also.

Blood count.

Red blood cells	3,240,000
Haemoglobin	70 per cent
White blood cells	5,300
Polymorphonuclears	85 per cent
Lymphocytes	13
Eosinophiles	2

A node was excised from the left axilla and from it an emulsion was made for guinea-pig inoculation. No trace of tuberculosis could be

found in the guinea-pig, however, when it came to autopsy. Examination of the urine for Bence-Jones protein was negative.

Autopsy. At autopsy there were found projecting nodules in the inguinal, axillary, and cervical regions. The lymph nodes about the iliac vessels and the aorta were greatly enlarged, and composed of firm grayish tissue, the largest measuring 6 or 7 cm. in diameter. The capsule of many of the nodes had been invaded, so that in places several were fused together into a single large nodular mass. These masses closely surrounded the aorta, and the vena cava was also embedded in tumor, to which its wall was in places densely adherent. The bronchial and mediastinal nodes were enlarged in the same way and fused into one mass. The psoas muscle on each side, and the lumbar vertebrae, were invaded by direct extension of the tumor from the retroperitoneal nodes.

Lungs. Dense coats of gray tumor tissue were found following the bronchi and blood-vessels into the substance of the lung, and also some button-like nodules on the pleura.

Liver. Weight 1450 grams. It contained numerous projecting nodules of a rather grayish-yellow, translucent appearance; in the region of the cystic duct there was a single large nodule.

Spleen. Weight 1820 grams. It was largely made up of clusters of firm, grayish, more or less discrete nodules of tumor. It showed also large areas of infarction.

Intestines. Normal. No enlargement of the lymphoid structures.

Bladder. There were distinct tumors in the muscular coat.

Uterus, vagina, ovaries, kidneys, adrenals, and aorta. Showed nothing noteworthy.

Bone marrow. The marrow of the lumbar vertebrae was fatty and yellow. In the marrow of the crest of the ileum, a single nodule was found.

Heart, stomach, rectum. Normal.

Microscopical examination. The structure of the tissue and the appearance of the cells is much the same in all the tumor masses. The architecture of the lymph nodes has been entirely lost; there are few blood-vessels and very little stroma, that present being mostly in the form of filaments dividing the tumor cells more or less into groups. The growth has invaded the capsule of the lymph nodes to some extent, but the separation, by the capsule, of the tumor cells within the node from the surrounding tissue, is still distinct. The cells show great variation in size, but average about 12 to 14 micra, though

some are fully three times the size of a lymphocyte; these are often angular and irregular in shape. The well preserved cells have an oval, slightly irregular, or round vesicular nucleus with a sharply marked nucleolus. The cytoplasm is wider than in a lymphocyte and stains a grayish-blue color. Mitotic figures in every stage are abundant. An occasional multinuclear element much larger than the predominating type can also be found after careful searching.

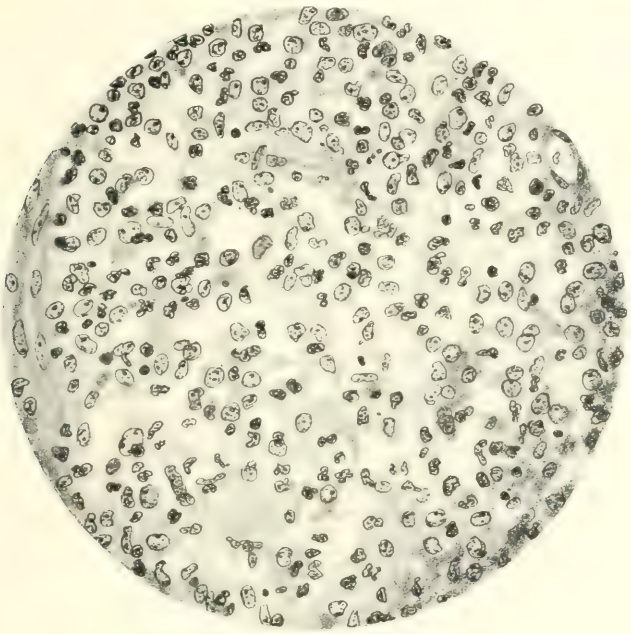

FIG. 2. CASE III

Note the great variation in the size and shape of cells, the presence of fragmented nuclei, the poor vascularization, and the lack of stroma.

Case IV. The patient was an Italian of forty-two, admitted to the Presbyterian Hospital in February, 1914, complaining of swelling of the right leg of about eight months' duration. Physical examination showed a firm mass the size of an egg in the right groin, nodular masses in the right lower quadrant of the abdomen, an enlarged spleen extending 5 cm. below the costal border, no enlargement of the liver, and enlarged nodes in the cervical and axillary groups.

Wassermann. Positive +++.

Spinal fluid. Negative.

Blood count.

Red blood cells	5,000,000
Haemoglobin	85 per cent
White blood cells	6,100 to 14,000
Polymorphonuclears	68 to 78 per cent
Small lymphocytes	18 to 26 per cent
Large lymphocytes	2 to 8 per cent
Large mononuclears	1 to 6 per cent
Eosinophiles	1 to 4 per cent
Basophiles	0 to 1 per cent

Urine. A faint trace of albumin and a few hyaline and granular casts.

Blood pressure, pulse, and temperature. Normal.

The superficial lymph nodes steadily enlarged, and became adherent to one another and to the surrounding tissues; great oedema developed in both legs and in the scrotum; the spleen and masses in the abdomen steadily increased in size. The liver was not palpable. A bloody effusion developed in the left chest. Anti-syphilitic treatment had no effect, either upon his general condition or upon the enlarged lymph nodes. Rectal examination disclosed a large mass nearly filling the pelvis. The urine showed no Bence-Jones protein.

Autopsy. In the cervical, axillary, and inguinal regions there were large masses of nodes matted together, none of which, however, was adherent to the skin. The mesenteric and retroperitoneal nodes were greatly enlarged, the largest masses being found just above Poupart's ligament on the right side. In the mediastinum and about the bronchi, the lymph nodes were distinctly enlarged, but less so than in the retroperitoneal chain. The enlarged nodes were generally well encapsulated, although adherent to one another. Nowhere was there a diffuse infiltration of the surrounding tissues. The pleura of the left lung contained large flattened tumor masses; the pleura of the right lung had similar flattened masses but much less extensive. The vessels and bronchi entering the lung were surrounded by coats of whitish tumor, but there were no other nodules in the substance of the lungs. The spleen weighed 2250 grams, the enormous increase in size being due to a great mass of nodules of the same pale gray, firm tumor tissue. A few of the nodules had a yellowish tint and were softer than the others; their average size was about 2 cm. Small masses were also found on

the diaphragm. Heart, aorta, liver, gall bladder, pancreas, adrenals, bladder, and prostate showed nothing noteworthy. The intestines were normal. There was no enlargement of the intestinal lymphoid tissue.

Microscopical examination. The structure of the tumor and the character of the predominating cells are here similar to those in case III, though the nuclei stain somewhat more deeply. In some places, an alveolar arrangement is very definite. The large cells which, in

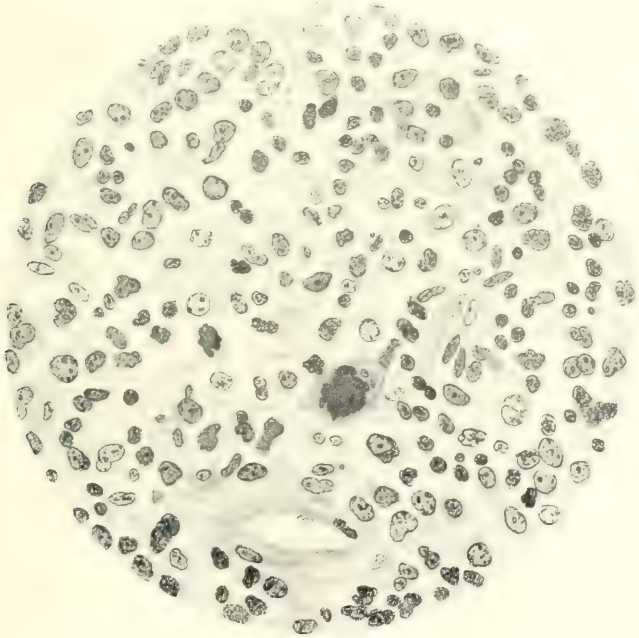

FIG. 3. CASE IV

The cells are very large and vary greatly in shape and size. Extremely large cells with irregularly shaped, deep-staining nuclei are common.

case III, were very scarce, are in this case numerous, almost every low power field having four or five. Some of these have several nuclei closely crowded together, others a single, large, convoluted nucleus. The largest of these elements measure 40 to 50 micra in diameter and have a nucleus in most cases staining very darkly and occupying two-thirds to three-fourths of the cell. The non-granular cytoplasm stains a grayish-pink color and is sharply outlined. These cells resemble

somewhat the megalokaryocytes of bone-marrow, although the nucleus occupies relatively more of the cell and is richer in chromatin. The bone-marrow sections show focal accumulations of cells quite the same as those found in the lymph nodes.

The similarity in these two cases (III and IV), is very striking in many particulars. Clinically, the following points of similarity should be noted:—One patient was forty-two, the other forty-four; in one the disease ran its course in nine months, in the other in fourteen months; in both the first lymphatic involvement noticed was in the groin; in both a marked enlargement of all the superficial lymph nodes followed within a few months; both showed enlargement of the liver and great enlargement of the spleen, as well as nodular masses in the lower abdomen; in neither was there a leukemic blood picture.

In their pathological anatomy the two cases are likewise very similar: the general lymphatic enlargement, the tendency of the enlarged nodes to become fused together into large masses without, however, diffusely invading their surroundings, as was the condition in the first two cases; the great enlargement of the spleen from the presence of discrete tumor nodules closely crowded together, the involvement of bronchi, pulmonary vessels, and pleura in a characteristic manner, the absence of any change in the lymphoid tissue of the intestines, and finally, the presence of discrete foci of tumor cells in the bone marrow without the presence of Bence-Jones protein in the urine. All these features were present in both cases. One of them did, however, show discrete nodular metastases in the liver and the urinary bladder, while the other did not.

The resemblance between the two is further borne out by the histological picture. The structure of the tumor tissue, and the size, shape, and staining affinities of the predominating cells are much the same in each. Case IV has, however, many more of the very large cells and these, moreover, are larger and have more darkly staining nuclei than the large cells in case III.

When we try to place these two cases in any of the recognized groups of primary enlargement of the lymphoid tissues,

difficulties are encountered at once. From the Kundrat lymphosarcoma they are distinguished by the following characteristics:

1. The early involvement of the lymph nodes throughout the body, in a manner which does not suggest a regional extension.

2. The formation, in great numbers, of discrete nodular metastases in the liver (case III) and spleen (both cases).

3. The much less marked tendency to grow out into the surrounding tissues, but rather to form large masses of nodes, adherent to one another.

4. The very different histological picture:—the greater average size of the cells and the variation in their size and shape, the more vesicular character of the nuclei, and the presence of very large cells which in one case have an appearance suggesting megalokaryocytes.

From pseudoleukemia or aleucocythaemic lymphatic leukemia these cases are readily differentiated by the type of splenic enlargement, the nodular metastases, and the different histological picture, these cases having cells bearing no resemblance to the lymphocyte.

From the leucosarcoma of Sternberg, assuming that there may be an aleukemic stage or form of this condition, the cases I have described may be differentiated:

1. By the fact that in leucosarcoma the splenic enlargement is due to a diffuse infiltration of the whole spleen by the abnormal cells, and not to the presence of clusters of discrete nodules.

2. The liver is affected in leucosarcoma in the same way as in leukemia, that is, by infiltrations.

3. By the absence in leucosarcoma of the very large cells found in the two cases I have described.

It is hardly necessary to point out that the histological picture alone of Hodgkin's disease is sufficiently characteristic to make it unnecessary to indicate other points of differentiation.

SUMMARY

In conclusion, then, the four cases included in this preliminary report fall into two groups. The first two have enough in

common with the Kundrat-Paltauf lymphosarcoma to be classed as such. The other two cases differ so essentially from lymphosarcoma, pseudoleukemia, and leucosarcoma that to place them in any of these groups seems unjustifiable.

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The family Koellikeriadae (Didymozoidae Mont.).

By

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With Plate 1–3.

The genus *Didymozoon* was created by Taschenberg, 1878, 1879 to comprise "worms elongated, sometimes kidney shaped or grown together in a ring with demarcated, often thread-like neck; without suckers. Pharynx spherical or oval, intestine present or absent. Testes forming a tube in numerous convolutions. Egg bearing canal extending in coils with the latter throughout the body. Genital orifice at the anterior body end. Vitellarium absent. The small oval eggs with chitinous shell present in millions in the uterus." The genus *Nematobothrium* later ranged with *Didymozoon* in this family by Monticelli and Braun is not concisely defined by van Beneden. Braun describes them as very much elongated Didymozoidae without intestine but with mouth opening and with genital pore at the head end.

These two genera make up the family Didymozoidae (Monticelli, 1888) defined by Braun as follows: Digenetic trematodes living always in pairs in cysts without separation of sexes. Anterior part of body narrowed into a neck, posterior part cylindrical or swollen into kidney shape; sometimes two individuals are grown together. Mouth sucker alone present; pharynx rudimentary or absent; intestine present or absent. Genital pore in front of mouth, terminal. Testis

a much convoluted tube accompanied by windings of the uterus. Eggs very numerous without filaments. Living on the outer surface of the body or in the mouth, branchial cavity or in the body of marine fishes.

As will be seen by anyone who reads the descriptions of the various worms which go to make up this family together with those of a number of other forms which have been given places among the Distomidae or Monostomidae, there still reigns the greatest confusion and it seems almost impossible to set the matter straight because in so many cases names have been assigned after only the most meager study of the worm and even without a single word of description. It is clear that some of the species belong not to the genus *Didymozoon* but to *Nematobothrium* while other genera have been established (*Koellikeria*, *Wedlia* by Cobbold) to receive forms which had previously been named *Monostomum* or *Distomum*. Of these genera which were proposed long before *Didymozoon*, Braun rejects *Wedlia* and retains *Koellikeria*. Both evidently contain forms which correspond with those described as *Didymozoon* and strict adherence to the rules of nomenclature should force us to use one of these names instead of *Didymozoon*..

Cobbold in 1860 described the genus *Koellikeria* as follows: „Sexus discretus. Corpus maris filiforme antrorsum clavatum, retrorsum sensim attenuatum. Os acetabuliforme orbiculare. Acetabulum ventrale sessile. Apertura genitalis inter os et acetabulum. Corpus feminae antrorsum filiforme clavatum, retrorsum subito increscens, reniforme. Apertura genitalis inter os et acetabulum. *K. filicollis* Cobb. = *D. okenii* Koell., *D. filicolle* van Beneden, *Monost. filicolle* Rud.

The same author described the genus *Wedlia* as follows, the description showing that it is like *Koellikeria* except in having only one sucker.

Wedlia: Corpus inerme, reniforme, lobatum; aliquando antrorsum attenuatum, apice incrassatum clavatum, retrorsum subito increscens. Os terminale acetabuliforme. Acetabulum ventrae mullum. Androgyna (?) apertura genitale infra os. Oviparum, ovulis non operculatis, avium incola et in cavo branchiarum piscium marinarum gemmatim in folliculis inclusum.

Wedlia bipartita Cobb.

Wedlia faba Cobb. = *Monost. faba* Bremser.

Probably none of these descriptions is absolutely exact since, as we shall see, the presence or absence of suckers seems to be a variable feature in these forms and rudimentary suckers or fading remnants of an atrophying and disappearing sucker make it difficult to decide whether there are two, or only one, or none. This appears to be the result of their perfectly inactive life in a cyst and although it is probable that they all originally had two suckers, these organs are now found in various stages of retrogression and their number can scarcely be used as the basis for separating genera.

With regard to the genital apparatus, close examination shows that while in one of the pair the female organs are highly developed and the male obsolescent, in the other the reverse is the case although in both there is a fading hermaphroditism. The outer form of the bodies corresponds with the predominance of the respective sex development.

Therefore specifications as to these points in the description of the genus have not the same decisive importance that they have in other genera and it seems on that basis that Cobbold's description of *Koellikeria* is about as accurate as the later ones and therefore by the rules of nomenclature since it is the first generic name which distinguishes these peculiar forms it should be accepted. There is nothing in the description given by Taschenberg for the genus *Didymozoon* which would justify the abandonment of the earlier genus, and his statement that there are no suckers is even less accurate than Cobbold's division of the group into *Koellikeria* with two suckers and *Wedlia* with one.

There is no good reason why *Wedlia* should not be used as the generic name except perhaps that *Koellikeria* appears first in the same paper.

It is therefore proposed to substitute the name *Koellikeria* for *Didymozoon* throughout and the genus may be briefly redescribed as follows:

Koellikeria (Cobbold, 1860).

Digenetic trematodes living in pairs in cysts. Hermaphrodite but sexually distinguishable by the outer form and by the predominance in each of the genital organs of one sex with obsolescence

of the others. Anterior part of body narrow, posterior part cylindrical or swollen into kidney shape: sometimes two individuals are grown together. Suckers rudimentary or very feebly developed. Both may be present or one or both may be absent: pharynx and intestine may be rudimentary or absent. Genital opening near mouth. Ovary and yolk gland long convoluted tubes meeting in a shell gland at beginning of a long tubular uterus. Eggs yellow and rounded. Testes saccular or tubular with long convoluted vas deferens. No muscular cirrus. Many forms are supplied with a nutritive vascular film from the host.

The situation may be presented most clearly by giving a brief summary of the descriptions of the forms which appear to belong in each of the long accepted genera *Didymozoon* and *Nematobothrium*.

Genus ***Koellikeria*** Cobb. (***Didymozoon*** Taschenberg).

K. (D.) thynni Taschenberg. (*Monostomum bipartitum* Wedl, Wagener etc.)

Wedl's description follows: Cysts 3—4 mm in size found on the gills of *Thynnus vulgaris* contain one or two peculiar monostomes. There is a pyriform head and reniform body connected by a long strand. Head measures 1,4 × 0.6 mm, the abdominal part 2 mm, the neck 2 × 0,8 mm. The smaller of the two worms which seems sexually immature has a much smaller abdominal part. Anterior sucker obliquely perforated, behind it the opening of the uterus which runs back into the abdomen. Thin grey cords seen there represent the ovary. Eggs yellow, brown, reniform 0,026 × 0,005 mm.

Taschenberg adds, pharynx oval, intestine present. The two individuals of one cyst grow together into a roundish structure with two heads. Length 6 mm × 4,5 mm.

Many others have described this worm, Wagener mentioning 3 types and Ariola separating 3 species which he assigns to 2 new genera *Didymocystis* and *Didymostoma* as follows:

Didymocystis reniformis (Wagener's second form).

Animals in each cyst similar to one another. The two regions of body sharply demarcated, posterior sac like, anterior filiform 3—4 mm long and enlarged anteriorly. Mouth small, terminal with double muscular pharynx but no sucker, short oesophagus and two

simple intestinal coeca. Genital opening below bifurcation, with genital canal. Parallel to this canal runs the uterus which opens near the mouth. Eggs bean shaped 16 × 10 μ. Animal 10 × 6 mm.

Didymostoma bipartitum (Wedl, 1855), (Wagener's first form).

Identical with *Didymozoon micropterygis* of Richiardi. Cysts with two dissimilar individuals, one large as a pea with two regions, filiform and globular; the other minute without posterior swelling. Mouth sucker present, but no pharynx; short oesophagus bifurcates in two simple intestinal coeca. Uterine opening near mouth. Eggs 21 × 11,6 μ. Small individual has mouth sucker and larger and longer neck. Measures 3,5 × 2 mm. The smaller is 2,5 mm in length.

Didymocystis wedli (Wagener's third form).

Cysts on gills contain two equal individuals with elongated body, the abdominal region of which is divided into two parts, a caudal recurved cylindroid and a larger anterior part indented by a groove from which the head emerges. No swelling of cephalic end, no mouth sucker, double pharynx. Oesophagus divides into two intestinal coeca. Genitalia as in other forms.

Apparently then at least three different species have been known by the same name but it seems doubtful that new generic names should be established on such slender basis as stated in Ariola's descriptions and I prefer to class all three as *Koellikeria*, especially since other species of this genus possess mouth suckers.

Koellikeria (*Didymozoon*) *scombri* Taschenberg.

Body elongated, rounded at both ends, neck only slightly narrower than the body. Pharynx spherical, intestine present. Opening of uterus on a little papilla et anterior end of animal. Length 18 mm. Cysts on the gill covers of *Scomber colias.*

K. (*D.*) *pelamydis* Taschenberg.

Body very long, slightly narrowed posteriorly and sharply pointed; neck thin, head widened, spoon shaped. Pharynx spherical. Intestine lacking. Length 30 mm. Cysts between gill leaflets of *Pelamys sarda.*

K. (*D.*) *sphyraenae* Taschenberg.

Body short, compressed, rounded posteriorly. Neck sharply marked off from body; head very little broadened. Pharynx oval,

intestine present. Length 15 mm, breadth 1.5 mm. Cyst under the mucosa of the mouth of *Sphyraena vulgaris*.

K. (*D.*) *auxis* Taschenberg.

Body long and slender, nearly rectangular, sometimes folded at the margins. Neck very sharply marked out from the body, thread-like. Pharynx oval. Intestine lacking. The two individuals in a cyst grow together sometimes through the union of anterior and posterior body ends into a ring. Length 12 mm. In cysts on the outside of the gill leaflets of *Auxis rochei*.

K. (*D.*) *serrani* Monticelli.

"This new species was found on the gills of a *Serranus fimbriatus* from Madeira; the same undescribed species I have frequently found attached to the gills of *Serranus gigas* of the Gulf of Naples. At present I give only a figure of this new species to ensure its recognition. The detailed description with anatomical remarks will be given in a forthcoming paper. The lengths of the cysts of this new species are 6—10 mm."

D. serrani is the first species of this genus found in fish of the family *Percidae*, the other known species inhabiting fishes of the families "*Scombridae* and *Sphyraenidae*".[1])

K. (*D.*) *pretiosus* Ariola.

A single cyst found on the gills of *Thynnus vulgaris* contained two equal individuals. Abdomen long flattened and ribbon-like, as in *D. sphyraenae* and *pelamydis*. There is a quadrilateral expansion at the union of neck with the body. Mouth sucker lacking. Length 11 mm of which 8 mm belong to the abdomen.

K. (*D.*) *micropterygi* Richiardi.

This paper was inaccessible to me but Ariola states that the form is identical with his *Didymostoma bipartitum*.

1) Such characteristic descriptions form the source of the confusion referred to above. No further description has appeared to my knowledge and we know nothing of this worm — not even whether it merits a separate specific name or not.

K. (*D.*) *exocoeti* Parona et Perugia.

This paper is also inaccessible but the form is the same as *Monostomum filum* Duj. and lives in the liver and in the eye of *Exocoetus exiliens*. It possesses a long thread-like body attached to the cyst wall, a mouth sucker and pharynx.

K. (*D.*) *benedenii* Monticelli.

A new and interesting species of *Didymozoon* found on the gills of *Orthagoriscus* probably same as that found by van Beneden but given this name. Nothing can be determined of the nature of this worm, especially since Maclaren who thought it identical with his properly described *Nematobothrium molae* was persuaded by Monticelli that it was a different worm. At any rate it is probably a *Nematobothrium*.

K. (*D.*) *filicolle* Rud., 1819. van Beneden, 1858.

Syn. for *Distomum filicolle* van Beneden, *Monost. filicolle* Rud., *D. okenii* Köll., 1849, *Köllikeria filicolle*.

Two different individuals in each cyst in the gills of *Brama raja*. The sac is not really a cyst as it opens into the branchial cavity. One individual is full of eggs, the other thin and delicate. The anterior part of body is narrow, slightly swollen anteriorly. Rest of body very large and rolls on itself. Buccal and abdominal suckers easily recognized. Intestine bifurcated — between the coeca a canal which opens behind the buccal bulb. This is the uterus partly filled with eggs and convoluted in posterior part of body. There are other tubes not yellow but milk white all belonging to the female apparatus. The other individual is like this in the anterior part but the body narrows behind and is elongated. There is a long tube beside the intestine but no eggs. Probably the male organs atrophy in one, the female in the other. Wagener has seen spermatozoa and eggs in the same individual and two of this kind in a cyst. There are worms which are more or less female or more or less male (van Beneden).

K. (*D.*) *tenuicolle* Rudolphi, 1819.

Syn. *Monost. tenuicolle* Rud. *Dist. affine* Dies. *Dist. decorynum* Dies. and probably *Didymozoon lampridis* Lönnberg. Worm encysted

in flesh of *Lampris guttatus* measuring 20 × 30 lines. Neck narrow. Abdomen full of vessels of three kinds two of which are brown. The drawings which R. gives make it clear that this is a characteristic *Didymozoon*.

K. (D.) lampridis Lönnberg.

From cysts in gills of *Lampris guttatus*. Worm 30—35 mm long paired in cysts but not united. There is a mouth sucker instead of a pharynx with radiate and circular muscle. No intestine present, the loss of intestine and weak development of musculature connected with the mode of life in cyst (Braun).

It will be seen that in no single instance is the anatomy of these worms at all completely understood but the external characters are such that it seems entirely justifiable to range them in one genus — *Koellikeria*. The following descriptions of other species recently met with may throw a little light on the finer structure and the relation of the worms to the allied genus *Nematobothrium*.

Koellikeria xiphiados n. sp.

In cutting up a sword fish (*Xiphias gladius*) a fisherman at Woods Hole came upon several cysts embedded in the muscles behind the gill cavity and containing large rounded masses which could be easily shelled out. He shelled out all of them and mutilated all but two so that the relations were much obscured, but in the material which he brought to the laboratory the U.S. Fish Commission, it could be seen that the cysts were thick walled and lined with a smooth white tissue. They measured 31 × 36 × 50 mm and two of them which lay close together had a common partition wall. They were situated just under the skin of the host the most distant one being 70 mm from the branchial cavity. From each there ran toward the gill cavity a cord made up of blood vessels which extended into the cyst and into relationship with the enclosed parasite in a peculiar way. There was no evident opening in the cyst wall through which communication with the outside could be established. The contents of the cysts were rounded or ovoid masses covered with a very thin, white wrinkled or smooth velvety membrane which ruptured at the slightest touch showing the parasite to be made up of irregularly triangular or wedge shaped lobules connected at the center by their apices. Into this mass there penetrated in every direction the vas-

cular film supplied with the arterial blood of the host through the cord described and drained by the vein of the same cord. This arrangement is almost like the vascular stroma of a tumor or like the circulation supplied to the foetus inasmuch as it evidently maintains the nourishment of the parasite.

The ovoid worms measured 24 × 28 × 40 m. There were in all five of them although one was reduced to a hardened brown shrunken mass which proved later to contain many recognisable eggs although the rest of the body was shrunken into a sort of mummified form. Otherwise they were all alike in form. Whether they lay two in a cyst or alone cannot be stated with certainty. On attempting to make out something of the structure of the animal it was found almost impossible to avoid tearing the fragile skin which formed the smooth outer surface and thus allowing the segments to pull apart. However, as will appear, these segments are not all of the same size and are not all continuously connected in the interior of the worm. Instead the vascular membrane entering between them at one point spreads out from the middle to lie in crevices among them in every possible direction. Any section of the mass shows these spaces containing a filmy connective tissue carrying the blood capillaries of the host.

At about that central point one may discover, by separating the segments, a small pointed white teat-like projection not more than 5 mm in length which proves to be the head. Ordinarily it is quite hidden down among the voluminous masses of the body, and whether it can stretch out so as to appear outside the sphere is difficult to say.

Little could be made out of the structure of the worm except by means of serial sections since the tissues were so excessively delicate and at the least touch ruptured to flood everything with eggs which appeared like yellow paint, so fine and so adherent they are.

A whole worm was embedded in celloidin and cut into thick serial sections which gave the topography of the internal organs. From another thin sections were made in various situations and the head was separated and cut into serial sections. From all these it was determined that the following conditions exist:

The pointed head and neck measure 5 × 1,4 mm and therefore do not form an elongated filiform structure such as has been described in other forms. At the base this neck spreads out into the lobu-

lations of the body which rise up all round it so as to hide it from view.

At the extreme point there can be seen an indefinite rounded mass which shows some muscle fibers and which is apparently the representative of a mouth sucker or pharynx. No distinct opening can be seen and no definite vestige of an oesophagus or intestinal tract can be traced away from it. Somewhat lower in the neck there is a faint outline of a tube which bifurcates and which in its turn may be the remnant of a digestive tract but no connections could be shown.

On the other hand, the uterus and vas deferens are extremely prominent and open together at a point beside the muscular mass.

The uterus is thickwalled and muscular and somewhat coiled even in the neck — it continues back into the root of the neck and disappears into one of the lobes of a body where, becoming wider and thinner walled it extends continuously through all the lobes to its origin in a small isolated lobule situated rather near the neck at the junction of the lobes.

Throughout part of the lobulations, although apparently not all of them, there runs the long tubular ovary. Careful study has been made to determine whether this tube branches into any of the lobes or runs continuously as one tube. In one place near the entrance into the uterus it seemed that it was joined by a branch but this is uncertain. Everywhere it has the appearance of being a single continuous tube. The same things are true of the narrow tubular vitellarium which takes little of the blue nuclear stain but has its own brown color. This courses through many lobes but is probably one continuous tube. It meets the ovary and gives rise to a single tube which after several convolutions during which it is thickly surrounded by the shell gland becomes the beginning of the uterus. There is no evident receptaculum seminis. The shell gland forms a relatively large mass of radially arranged cells and from it the uterus runs back to enter one of the general lobes. The lobule in which the shell gland lies and in which this junction of the various canals occurs contains no other organs and is rather separated from the rest although continuous with them at either end. Its skin is very much corrugated. The uterus in its main portion is thin walled and distended with huge numbers of eggs which are yellow and rounded and measure 0,06 mm in diameter.

The vas deferens which opens at the termination of the pointed

head without any special muscular reinforcement is convoluted and rather bulbous in this part; posteriorly it divides into two branches which extend into two of the lobes and expand into plicated sacs — the testes. Not all the lobes contain testicular material and in the two which do the organ is evidently a continuous tubular sac. Spermatogenesis may be observed very clearly in all stages.

No excretory system could be clearly traced although there are many thin walled spaces and canals which doubtless have this function. Practically no musculature is found anywhere in the body except in the walls of the uterus. The whole mass is composed of a soft watery parenchyma of large cells and large spaces condensed into a firmer sort of connective tissue in the central mass where the lobules are held together. The skin is delicate and unarmed but provided with the usual cuticular cells.

It is most difficult of all to understand how the body acquired this peculiar shape and indeed to learn precisely what the relation of the parts is.

It was stated that the outer surface looked smooth and relatively white but that it readily cracked and fell apart revealing lobules. In section it is seen that the outer covering is not continuous over a smooth surface but that while it covers several lobulations it dips down between the main ones. Then, too, a quite similar cuticular covering is found upon lesser lobulations which are hidden in the main mass and upon the inner side of the various parts which go to make up the main lobulations. It is as though there were many folds or lobules which had fused in groups wherever they presented externally so that while over their outer surface, skin and parenchyma became continuous the inner parts remained discrete each covered there with skin so that a whole series of little crevices or caverns arose between these lobules roofed over by the fused outer part. It is into these crevices everywhere that the vascular film from the host finds its way and brings nutriment and oxygenation.

But all the lobules seem to fuse together at the centre into a sort of common core of denser tissue into which the vasa deferentia and the uterus run to enter the neck. The mass which contains the shell gland and the connection of ovary, vitellarium and uterus lies in this central part and indirectly connected with the central core.

It will be seen from this necessarily imperfect description that this worm differs in several respects from those already described.

It is lodged in cysts embedded deep in the muscles and not, as in most other cases in relation with the gills. It is definitely hermaphrodite both male and female organs being well developed. Whether two worms or only one live in each cyst we cannot state with absolute certainty since all were turned out of the cysts by the fisherman who could give no precise information about it. From measurements it would seem, however, that two of the worms might have been accomodated in each cyst.

The neck is remarkably short and practically no trace of suckers nor of digestive tract remains. The peculiar invasion of all the interlobular crevices by a vascular film from the host is similar to that seen in the form of Nematobothrium to be described and in another form of *Koellikeria*. It probably occurs generally although it is rarely mentioned. Similarly the reniform or bean shaped body so commonly found might prove to show lobulations such as are seen here, if carefully studied. From the arrangement of the uterus, ovary, vitellarium etc. it seems probable that this curious form is in this case at least not due to the mere swelling of the posterior end of the body but rather to the complicated coiling into a compact mass of what was originally a greatly elongated body with secondary fusions such as to consolidate the whole into a compact mass and even weld together the outlying parts into a smooth surface. The form next to be described from *Haemulon* presents a similar process in a simpler stage.

How the eggs escape from these cysts to the outer world is a problem which we cannot solve with the facts at our disposal. From somewhere near the branchial cavity a cord-like band was traced through the muscles to each cyst and this cord was found on section to contain a large artery and several veins which supply blood to the film described as penetrating the crevices of the worm. The suggestion has been made that some channel might exist along this cord through which the neck of the worm could extend to discharge eggs or through which the eggs themselves might pass into the branchial cavity, but no such canal was demonstrated. One specimen was found dried up into a mummified mass in which eggs were recognisable and this has also been observed in another form. It seems possible, therefore, that sometimes the eggs may never escape unless the host dies and disintegrates, when they may pass into some intermediate host.

Diagnosis: *Koellikeria xiphiados*. Large worms found encysted

in muscles of *Xiphias gladius*. Neck short, suckers, pharynx and digestive tract practically absent. Uterus and vas deferens open together at cephalic extremity. Body forms a lobulated mass with extensive fusion of lobules. Nutritive vascular film from host extending everywhere among these lobules. Eggs innumerable round and yellow. Shell gland bulky. No receptaculum seminis. Dimensions $40 \times 28 \times 24$ mm.

Koellikeria scomberomori n. sp.

On October 1, 1911 there were found in the stomach of the Spanish Mackerel (*Scomberomorus maculatus*) 5 worms 4 of which were loose while 1 lay in a cavity or cyst in the wall of the stomach from which it could be pulled out. These were kidney shaped and measured $1{,}75 \times 1{,}08$ mm with a slender neck which protruded 0,12 mm. No exact study could be made since none of the worms were cut into sections, but coils of the uterus full of eggs, the tubular ovary and vas deferens could be seen shining through the body wall. The ova measure $0{,}014 \times 0{,}01$ mm.

In the very delicate neck the uterus and the vas deferens can be seen extending quite to the tip of the cephalic extremity. There is a small muscular mouth sucker and a very definite muscular pharynx there but no alimentary tract could be definitely made out.

Further study of this parasite will be necessary when better material is obtained before its systematic position can be more definitely established but there seems no question of its belonging to this genus and its size and shape and the form of the neck, which shows no cephalic enlargement, seem sufficient to distinguish it from any of the described forms.

In the same fish there were found on another occasion, August 15, 1912, four small pear shaped cysts of a bluish or lead color firmly attached to the gill filaments. They measured 5×3 mm and at the center of the larger end there could be seen a small dark spot which seemed to be an opening. On teasing out the contents of one cyst it was found to contain two worms of an orange color with much black material in several parts of the body. Serial sections showed the cysts to be very thick walled and the contents to be almost entirely necrotic and loaded with black pigment. Many eggs of oval form measuring 0,01 mm could be seen but no outlines of the organs remained visible. In this case we venture nothing further but await more material for a more satisfactory study. The

parasite is probably a different form from that which we have named *K. scomberomori.*

Koellikeria haemuli n. sp.

On the pseudobranchs of *Haemulon flavolineatum* (Yellow Grunt) there were found (New York Aquarium) two reddish prominent masses which measured about 6 × 4 × 2 mm and which were intimately attached to the underlying bony tissues. In removing them they were unfortunately somewhat torn so that certain imperfections appeared later in the serial sections. Surrounded tightly by a fibrous capsule the enclosed worm was not to be everted but seemed rather to be embedded in a meshwork of fibrous partitions. Many elliptical yellow eggs were sifted off from the torn surface and these were found to measure 0,2 × 0,15 mm. Since it was impossible to isolate the worms one cannot give an accurate impression of their external form but from a reconstruction of the serial sections its appears as though a very much elongated cylindrical worm, or more probably two, were coiled together in loops and irregular twists, invested everywhere with the tissues of the host and in many places adherent or even partly fused together. This idea although not absolutely proven is supported by the fact that each loop or fold in every section contains one cross section of each canal represented and further by the fact that one may trace any canal such as the uterus through fold after fold in succession.

Two of the encapsulated masses were found and cut into serial sections. They differ somewhat in structure but are unquestionably of the same species. The difference in structure depends upon the fact that in one mass the female organs of reproduction are most conspicuous while in the other the male organs overshadow the female. Elements of both, however, are present in each case.

In such a complex tangle it was essential to determine first whether two worms were concerned in each mass or only one, and even now after the most painstaking study of these two series we are not absolutely certain, chiefly on account of the imperfection of the specimens but partly on account of the extreme intimacy of adhesion of the folds which obscures the outlines.

In the first series only one head has been found with terminal pharyngeal bulb, oesophagus and uterine orifice. In the second

series a quite similar narrow prolongation was found with uterus, intestinal coeca etc. but the end was broken off before the actual head was reached. Nevertheless the structure was so similar to that of the head in the other series that there seems no doubt that this is the head of one worm. In this second series another head was found with pharyngeal bulb, oesophagus and a strongly developed vas deferens filled with spermatozoa. Two worms exist, therefore, in the second series: possibly only one in the first. The series will be described separately.

The first series: The head and neck are extremely slender and tapered anteriorly. Where the neck passes into the general tangle of folds of the body it becomes much wider and quite dense in structure. The mouth is terminal, guarded by no sucker but opening directly into an elliptical pharyngeal bulb. From this the oesophagus runs a short way and bifurcates into narrow, very thinwalled intestinal coeca which run far back into the body of the worm but do not branch again. The uterus, which is extremely narrow at its termination, opens just behind the pharynx. In the neck it is very muscular and this thick wall can be traced far back into the body. No trace of the vas deferens can be seen there. A short way behind the bifurcation of the intestine there is a minute but quite distinct ventral sucker. This is almost embedded in the parenchyma but does not open on the surface. It is extremely weak and small and is rather indistinctly outlined.

The nervous system is visible in its coarser features in the form of the usual pair of ganglia about the oesophagus. The excretory system in the form of one or two thinwalled tubes appears in the neck and courses through the rest of the body.

The main bulk of the worm is occupied by the female organs of generation which are arranged as follows. Extending back from the anterior orifice the uterus widens and courses along through every fold, twisting and turning so that in some places it is cut two or three times, in others only once. It runs back through the whole body once as a rather straight tube exactly as in *Nematobothrium sardae* and then in coils comes forward again. At one point in the body all the uterine coils are pushed aside by the great mass of the shell gland and within this there takes place the union of the portions of the reproductive system. The ovary which is long and cord-like lies in the anterior part of the body. The vitellarium, also long and cord-like or tubular, extends through the posterior part.

They meet almost at a point in a thickwalled tube in the midst of the shell gland from which starts the narrow uterus. No receptaculum seminis nor Laurer's Canal could be found. At first the eggs in the uterus take a bluish stain and this continues for some distance finally giving place to the more mature ones with thick yellow shell. It may be remarked that in this worm the large mass of radiating cells which forms the shell gland occupies the whole width of one loop of the worm and is very conspicuous. It is traversed also by one strand of the mid portion of the uterus and by a canal which seems to belong to the excretory system. It is curious that the portion of the body just behind this through which the beginning of the uterus courses from the shell gland contains in addition only the straight strand of the uterus and one of the vitellarium. It leaves the general mass of loops and after a little returns again and enters into their midst.

In the more anterior part of the body there are masses of testicular substance but theier outline is very indefinite and difficult to trace. No vas deferens can be found and although there are certain loops whose content seems to be chiefly testicular tissue, which project from the inner surface of the mass it is impossible to reconstruct these organs clearly. Since little slender masses containing this tissue alone project sometimes from the edge of the section one gains the impression that they may be part of another worm with essentially male characters but no attempts to trace these were successful in anything more than losing them in the general mass — nor was a second head discoverable.

On the whole, then, in this mass was found a tangled coiled worm with highly developed female organs of reproduction opening near the mouth and ill defined testicular structures without recognisable connections. No second worm could be definitely made out.

In the second series the general appearance is at first sight the same. Abundant coils are seen to contain wide uterine canals filled with eggs and strands of the ovary and vitellarium but even the most careful search fails to reveal the mass of the shell gland which is so conspicuous a feature of the contents of the other mass. On the other hand the testicle is extremely well developed and sharply outlined and the vas deferens with its connection becomes very conspicuous.

Beginning in the mass of coils most distal from the broad attachment of the cyst is a distinct head closely resembling that

seen in the first mass in that the mouth opening is terminal guarded by a pharyngeal bulb and extending into a short oesophagus which quickly bifurcates. The nervous ganglia are very distinct and at their level just behind the pharynx is the opening of the vas deferens which is not provided with any especially muscular cirrhus. Traced back through the neck of the worm the vas deferens continues as a thickwalled tube and is spirally twisted. No uterine canal is visible. No abdominal sucker is found although an indefinite pink staining mass with numerous scattered nuclei is visible at about the point where it was found in the other worm. After a quite long course during which it passes back into the central part of the tangled coils without showing any evidence of the presence of an accompanying uterus, there appears a lateral pear shaped sac which is filled with spermatozoa and which from its connection with the vas deferens must be regarded as a seminal vesicle or reservoir. From that point the vas deferens becomes connected with the abundantly folded and convoluted testis. It has not been possible to make out with certainty whether in each worm all these testicular masses are connected. In the other cyst there were numerous disconnected masses. In this one those connected with the vas deferens appear to be continuous but other separate testicular masses appear in the field of the microscope in other coils and probably belong to the second worm entangled in this mass.

At the other edge of the section, that is in the area of attachment where the worm was somewhat torn there is a neck-like elongated structure containing the muscular uterus and two intestinal canals. This was broken before the extremity was reached but it corresponds exactly with the neck found in the first cyst and must be regarded as belonging to a second head. The uterus is muscular and can be traced back as a thickwalled tube through coil after coil in the main mass. It is full of eggs. No vas deferens is found in this prolongation nor any ventral sucker. Probably, however, this latter would have been in the part which was broken off. The rest of the coils which seem to belong to this head contain the large uterus with many eggs, ovaries and vitellarian cord but as mentioned before no definite shell gland nor any place at which these canals form a conspicuous union could be found although the whole series was searched carefully. Such a connection must be present but it must be very different from that seen in the other mass for otherwise it could not possibly escape attention.

From all this it is seen that peculiar conditions prevail in this species. The worm is a cylindrical elongated form which is coiled abundantly in a meshwork of connective tissue. In one specimen there is a pair, in the other as far as we can see only one individual. The single individual is a well developed female with obsolescent male organs. In the pair there is one with very distinct and highly developed male characters and obsolete if any female characters, and another with rather poorly developed female characters and obsolescent testicular masses without any trace of vas deferens.

The name *Koellikeria* is retained for this transitional form, although apparently the worm is even more closely allied to the genus *Nematobothrium* than to the genus *Koellikeria* if one may judge from its body form and from the arrangement of its organs which is precisely that of the *Nematobothrium*. It furnishes an especially brilliant example of the degeneration and disappearance of organs under peculiar conditions of life and possibly represents a transition toward the monoecious state.

How cross fertilization has occurred in these specimens it is difficult to surmise for the efficient male is enclosed in a cyst at a distance from the efficient female and has only a rudimentary female in the same cyst to fertilize while the female is isolated except for its own rudimentary male apparatus.

The wall of the cyst is more fibrous and less vascular than that of the *Nematobothrium sardae*; nevertheless there are bands and filaments of highly vascular character which run between the coils and doubtless supply them with nourishment. No distinct opening for egress of eggs etc. could be made out, but this point must be restudied when fresh material is found and sectioned without tearing of the attachment. Still the head bearing the opening of the vas deferens lies just under the perfectly intact outer wall of the capsule as though at least its projection through any external opening was unnecessary and further the fact that the opposite side of the mass where both the heads containing the uterine openings lie is the side of firm attachment to the bone so that it seems extremely unlikely that any opening occurred there. The escape of eggs to the outer world must therefore in all probability be through rupture of the cyst or its digestion after the death of the fish by some intermediate host.

Genus *Nematobothrium* van Beneden 1858.

The genus *Nematobothrium* established by van Beneden contains up to the present only 3 species if we except the forms *benedenii* and *taenioides* of Monticelli which have been referred to *Didymozoon*. These latter worms are merely mentioned and nowhere described in detail so that it is impossible to determine their exact position. Stossich who has studied *D. taenioides* describes it as follows: body very long, cylindrical, tapering toward the anterior part. The pharynx is small, subglobose and provided with a very long narrow oesophagus which divides into two narrow intestinal coeca. The uterus opens anteriorly at the extremity of a little prominence near the cephalic end of the body and contains thousands of very small eggs with thick smooth shell of a deep yellow color. It forms cysts, sometimes quite voluminous, in the muscles of *Orthagoriscus mola*. The figure shows no ventral sucker. This description seems to apply rather better to a member of the genus *Nematobothrium* than to a *Didymozoon* even though Monticelli definitely decides upon the latter designation.

The 3 species which are rather more clearly described are *N. filarina* van Beneden, *N. guernei* Moniez and *N. molae* of Maclaren but these descriptions too leave much to be desired.

Nematobothrium filarina found lodged in cysts in the thickness of the skin of the branchial cavity at the shoulder girdle in *Sciaena aquila* is described by van Beneden as a worm 1 metre long. It lives in pairs tangled together in the cavity of the cyst which has no connection with the outside. The body is long, round, soft and folded with no segmentation. It is not free in the cyst but lodged in a mantle which clings tightly. At one end there is an excavation which is not a sucker: at the other end, which is more truncated, there is also an excavation of different form. There is no ventral sucker. No trace of intestinal canal could be found, but it is thought that it may have existed before the great development of the genitalia and disappeared as in *Filaria medinensis*, *Gordius* and *Distomum filicolle*. Generally there are two worms enlaced, recalling *Distomum filicolle*, the thickest being rolled round the slender one which has less numerous eggs which are not yellow like the eggs of the larger one. There is a large tube with contractile walls ending blindly and disappearing in contraction. It must be the excretory canal and permits one to distinguish the posterior end.

The anterior end is pointed and very mobile. No buccal orifice is recognisable. Another tortuous canal is filled with eggs. The ovary oviduct or uterus is, a single long tube making four or five turns through the body, all full of eggs which distend the body in part of its length. Eggs very small, oval, yellow and extremely numerous. They often contain an embryo bent on itself.

N. guernei was found by Moniez attached by one or both extremities in the muscles of the inferior maxilla of *Thynnus alalonga* the rest of the body being free. In another case they were free in the intestine or caught in the mucosa, and in still others encysted in pairs on the gills in cysts about the size of a pea or bean. Those in the muscles measured 30—50 cm in length, the body elongated, striated almost as though segmented, sometimes round, sometimes flattened. There is a large tube opening posteriorly and stretching through the whole body and seeming to open anteriorly in the mouth with walls like those of the digestive tube of a nematode. Anteriorly there are two distinct sexual orifices superposed, a "poche péniale" prolonged by a spermiduct which separates into two immense testicular tubes. From the vaginal orifice goes an extremely long folded oviduct which prolongs itself into an ovary of the same general character. The water vascular system opens at the posterior extremity and is prolonged as a thick walled tube without ramifications to the anterior part of the body. No other organs were discoverable. Moniez thinks it possible that the smaller worms (3—15 cm) which were found in the intestine, the encysted and the intramuscular forms may represent alternations of generations.

N. molae is described by Maclaren from the gills of *Orthagoriscus mola* where it occurs encysted in pairs. It resembles *N. filarina* closely except in the possession of a small ventral sucker. The worm is 7 cm long by 2 mm in thickness, soft irregular in form with a threadlike anterior portion and a blunter posterior end. The mouth at the anterior end opens directly into the pharynx without a sucker. The genital opening lies on a prominent ventral papilla about 0.6 mm from the mouth. The body is enveloped in a thin sheath. There is a rather long oesophagus which divides into two short intestinal coeca lined with peculiar amoeboid cells. The excretory canal is a single irregular canal opening posteriorly and bifurcating anteriorly at the level of the ventral sucker. There are two testes of long tortuous tubular form stretching through the body which taper into vasa deferentia and unite into a bul-

ous seminal reservoir. There is no definite penis although the end portion of the canal may be everted. The long tubular ovary twists through the whole body. Its connection with the uterus was not seen. The uterus is a roomy canal which runs in loops through the hinder part of the body turning forward to open directly behind the male opening. Enormous numbers of eggs are found in it. The ripe eggs measure 0.02 × 0.015 mm and are without filament. Two tubular vitellaria accompany the testes and uterus in their windings.

Maclaren restates the diagnosis of the genus *Nematobothrium* van Beneden as follows: much elongated distomidae with small pear shaped pharynx, no mouth sucker. Mouth at anterior point: small ventral sucker not far from mouth; simply bifurcated intestine: excretory canal bifurcated at anterior end but without anastomosis over pharynx: Hermaphrodites: ovary long tubular: 2 long tubular vitellaria; eggs without filament: 2 long tubular testes without special penis: Genital canals open separately on a papilla somewhat behind the mouth; Laurer's canal? The animals live in pairs encapsulated in the gills or in the flesh of sea fish.

N. sardae n. sp.

On the gills of the Bonito (*Sarda sarda*) I found (July 8, 1913) in the Laboratory the U. S. Fish Commission of Wood's Hole a few yellow cysts which were lodged between the layers of the gills. They were flattened and measured 9 × 4.50 × 1.50 mm and were found to contain two worms which although tangled together are quite separate and unattached to one another. When disentangled and laid out straight these worms measure about 34 × 1 mm and are quite yellow. The mouth at the anterior end is provided with a fairly strong sucker but no abdominal sucker could be found after the examination of four or five worms mounted in toto and the serial sections of two pairs.

The anterior part of the body is rather suddenly narrowed from the rest but is flattened out somewhat for a short distance behind the head and widened accordingly. The terminal mouth sucker is quite strong and muscular. It opens directly through the more feebly muscular pharynx into the oesophagus. This after a very short course passes into a most curious single intestinal canal, which is surrounded by a thick bushy mass of cells so as to be a very conspicuous object. Running almost straight at first it soon

assumes a very wavy or zigzag course, the surrounding cells becoming more compactly and closely applied about it. Even where the mantle of cells is thickest the lumen can be seen shining through as an empty tube. It extends back only a short way, not more than four or five millimeters to a point about midway along the stretch of the testes where it becomes invisible. In the section it is seen, however, that it does not end here but passes into an extremely thinwalled tube. In spite of our best efforts we have not succeedel in tracing this continuation backward nor determining absolutely whether it divides or not. This is partly because its wall loses any distinctive character and becomes exactly like that of the wide and tortuous excretory canals which are present everywhere throughout the length of the body.

The anterior or mouth sucker is terminal and surrounded by no lip or prolongation. It is quite strong being provided with an inner and outer layer of circular as well as the main mass of radial fibers. Numerous large cells are embedded in this muscle and there is a fairly thick cuticular lining which passes on into the pharynx. This has a wall made up of a loose network of radial fibers and also shows large cells embedded in it. The oesophagus is a thin walled tube lined by a smooth membrane. Possibly the whole succeeding tube should be regarded as the oesophagus if it should ever prove that the thin walled sac at its end is bifurcated or that it represents the definite intestine. The lining membrane gradually thickens as we pass into this convoluted tube and becomes closely set with cilia but no lining epithelial cells are to be seen. Outside this membrane, however, the cells are ranged in radial strands closely packed about the canal but fraying out a little externally. They are narrow elongated cells with very definite nucleus and are at least ten or twelve deep all round the canal. It is hard to know exactly how to explain their presence and what function to ascribe to them. At the lower end these cells fade away and the ciliated lining disappears. — There is left only the most delicate membrane in which cellular structure cannot be made out clearly.

The nervous system consists of a pair of ganglia with commissural connection just behind the pharynx and several rather stout nerve trunks which run to the anterior end of the body and backward.

The excretory system appears to be composed of two large thin walled tubes which run from one end of the body to the other.

They twist about among the uterine coils so that in every section they appear repeatedly, cut in various directions and containing a faint granular coagulum. In spite of protracted effort, however, we could not determine where the orifice is situated.

Almost the whole body of the worm is occupied by closely twisted coils of the extremely long and wide uterus which is filled with yellow eggs. This gives the general yellow color which shines through the wall of the cyst. Among these coils one even wider straight length of the tube can be seen to stretch forward to the anterior end of the body. Among them, too, especially throughout the posterior portion there are mingled many coils of a narrower tube which is of a deeper brown color and in the more anterior part similar coils of another tube which appears rather white in the unstained worm but takes a bright nuclear stain which makes it conspicuous in the stained specimen. This proves to be the ovary, the brown tube in the posterior part the vitellarium. At the junction of the anterior and middle thirds of the body there is a space among all these coils in which one can make out a pear shaped seminal reservoir, a voluminous shell gland and the junction of all the tubes. The ovary lies in front of this space, the vitellarium behind it. The uterus opens abruptly in an almost unguarded slit just behind the mouth sucker at a point immediately behind and adjacent to the opening of the vas deferens. Studied in serial sections the following relations are found to prevail. The ovary is a pratically solid cord of great length and making many folds — it is tubular only in the sense that the central cells are less compactly placed than the outer ones. It reaches the point mentioned above without narrowing appreciably and joins a narrow rather thickwalled canal which receives at the same point the neck of the seminal reservoir and the narrowed channel from the vitellarium. There is no Laurer's canal. The seminal reservoir is a rounded sac bent on itself and tapering into a rather long neck. It is always found closely packed with bundles of stiff looking spermatozoa. The vitellarium seems to be a single convoluted tube which extends back with the uterus to the extreme posterior end of the body. It is lined with cubical or pyramidal cells which contain many highly refractive brown granules and the smaller cells which fall off into its central part constitute the yolk. They are indeed extremely small. These three ducts join at a point where they are surrounded and imbedded deep in a large conspicuous mass of cells radially arranged which one

must regard as the shell gland. This gland is developed to a very great extent probably because of its task of furnishing shell material to the myriads of eggs which collect in the uterus. The combined channel twists about as a very narrow canal through a great deal more of the shell gland and finally emerges toward the posterior end as a gradually widening tube filled with eggs which are at first small and polygonal and do not show perfectly their completed form nor their finished shell. It is only after many coils that the eggs assume their definitive appearance. The course of the uterus after this has been described. In this species it nowhere becomes so bulky as to distort the cylindrical form as described by Maclaren for *N. molae*. It opens on a little hillock just behind the mouth sucker and the terminal portion though surrounded by a few nucleated cells resembling those of the skin is not provided with any special musculature. The testis is single though much folded and twisted in its posterior part. It begins anteriorly as a sausage-shaped structure at about the point where the neck of the worm begins to widen out into the body and runs backward for a distance of only about two or three millimeters. Anteriorly it gives off a convoluted vas deferens which runs beside the uterus to practically the same point just behind the mouth sucker. There it dilates a little into a bulbous extremity which then narrows to pass into a pear shaped sac which opens again on the apex of a little hillock. This sac has at most a delicate layer of circular muscle fibres surrounded by a number of closely arranged deeply staining cells, but it is lined by a layer of stiff recurved hairs. It can probably be everted to act as a penis. The testis shows a compact outer layer of cells but in the interior the cells are grouped in clusters separated here and there by sheaves of straight rigid spermatozoa.

There seems to be some indication of a separation of the sexes in this form, for although each worm possesses both male and female organs in full development as far as can be seen from study of the testis, ovary, shell gland etc. the terminal portion of the uterus with the uterine outlet is well developed and conspicuous in one of the pair but scarcely traceable in the other. On the other hand the vas deferens with its bulbous seminal reservoir and the sac which serves as a penis is very striking in the other worm of the pair while in the first one in which the uterine opening is so evident no such sac can be found nor can the vas deferens be definitely traced to an outlet. It is quite empty of spermatozoa in its cephalic

portion and escapes from view before it reaches its point of outlet. Better sections might still reveal these fading terminations but in the two pairs studied in series the contrast was very striking. It may conceivably be the result of the functioning of one worm as a male and the other as a female during which the remaining sexual function of each was held in abeyance. It reminds one of the statement of VAN BENEDEN in regard to *N. filarina* that the smaller worm of the pair has less numerous eggs than the larger and that these are not yellow as they are in the large one.

The eggs are very small (about 0.01 mm) elliptical: some of them contain stainable material while others seem to be mere empty shells. They are present in enormous numbers.

The skin is delicate and thin, supplied as usual with many secreting cells. The body musculature is extremely poorly developed — indeed except in the anterior end of the body it can hardly be made out at all. Even about the head the longitudinal and oblique fibres are extremely delicate. The parenchyma in the head end is composed of large swollen cells with small nucleus much as described by MACLAREN. In the posterior parts it is scarcely evident.

The worms seem to arrive at complete sexual maturity within the cyst and evidently copulate there. No mode of egress was demonstrated. They are not covered with a sheath such as VAN BENEDEN described but lie quite free in the cyst.

The outer wall of the cyst is composed of a pretty dense fibrous tissue within which there is developed a most highly vascular lining. From this lining there run partitions and films of exceedingly richly vascular tissue among the folds of the worms evidently to supply them with oxygenated blood. In section one sees this membrane as a closely beaded band each bead representing a distended capillary of the host carrying fresh blood. It might be imagined that in that way an adequate supply of nutriment and oxygen could be brought to the worm even if it made no attempt to actually suck blood from this film — an idea which the character of its alimentary tract and its emptiness seem to go far to prove.

This form seems to belong without doubt to the genus *Nematobothrium* although it differs in so many points from *N. molae* which MACLAREN has described and from the forms described by VAN BENEDEN and MONIEZ although their descriptions are rather too meager to allow of accurate comparison.

These are trematode worms resembling the genus *Koellikeria*

in living in pairs enclosed in cysts, a mode of life which brings with it modifications and retrogressions of various organs. They are distinct from *Koellikeria* in the form of the body which is long and cylindrical but similar in the possession of a long cord-like ovary and vitellarium and in the position of the genital opening at the cephalic extremity. I should prefer with Braun to regard *Nematobothrium* as a part of the family *Koellikeriadae*, separate from the *Monostomidae* or *Distomidae* and define the genus as follows:

Nematobothrium van Beneden, 1858.

Trematodes of greatly elongated form living in the mature state in pairs in cysts in gills or flesh of sea fish. Digestive tract in various degrees of retrogression. Hermaphrodite but tending to retrogression of one sex in each. Genital openings directly behind mouth at cephalic extremity. Ovary and vitellarium in form of long coiled cords, shell gland and seminal reservoir well developed, uterus forming extremely long coiled tube. Single testis and vas deferens, rudimentary penis.

The forms recognized up to the present are:

N. filarina van Beneden	*Sciaena aquila*
— *guernei* Moniez	*Thynnus alalonga*
— *molae* Maclaren	*Orthagoriscus mola*
taenioides (?) Monticelli	„ „
— *sardae n. sp.*	*Sarda sarda.*

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We wish to express our gratitude to Dr. B. H. Ransom of the U. S. Dept. of Agriculture who kindly sent us photographic copies of some of the less accessible literature.

Explanation of plates.

m. s Mouth sucker
ph Pharynx
i Intestine
f. g. p Female genital pore
m. g. p Male genital pore
v. d Vas deferens
g Supraoesophageal ganglien
v. s Ventral sucker
ut Uterus
s. g Shell gland
ov. d Oviduct
oot Ootype
vit Vitellarium
ov Ovary
ves. sem Seminal vesicles
oes Oesophagus
Ex. c Excretory canal
r. s Receptaculum seminis

Plate 1.

Koellikeria xiphiados.

Fig. 1. Portions of cyst in the muscle of the sword fish from which worms have been removed.

Fig. 2. Whole worm. Lobules spread apart to show projecting head.

Fig. 3. Section of portion of one lobe showing coils of uterus, testis and vitellarium. The cuticular covering extends over all lobules and between them is the vascular film from the host.

Fig. 4. Longitudinal section of the head showing muscular pharynx, uterus and vas deferens.

Fig. 5. Transverse section of vascular cord running through muscles of the host to the cyst.

Koellikeria scomberomori.

Fig. 6. Sketch of whole worm showing mouth sucker and pharynx and arrangement of organs in body.

Plate 2.

Koellikeria haemuli.

Fig. 7. Section taken through the whole encysted mass showing uterine and ovarian coils as well as some testicular folds. Cyst wall sends septa between the coils of the worm.

Fig. 8. Head showing mouth sucker, pharynx, supraoesophageal ganglia, uterus vas deferens and intestine.

Fig. 9. Section through several lobes showing the shell gland with the projecting junction of oviduct, vitellarium and uterus.

Fig. 10. A coil of the relatively male worm showing testis, vas deferens and seminal vesicles.

Nematobothrium sardae.

Fig. 11. Whole worm.

Plate 3.

Fig. 12. Head and neck showing mantle of cells about intestine.

Fig. 13. Longitudinal section of mouth sucker, pharynx and oesophagus.

Fig. 14. Longitudinal sections of the heads of the worms forming a pair, showing the inequality of development of the sexual apparatus.

Fig. 15. Longitudinal section through cyst wall, three coils of the worm and the vascular film.

Fig. 16. Portion of the whole worm showing junction of ovary, vitellarium, receptaculum seminis and beginning of the uterus in the midst of the shell gland.

Note. Since returning the proof of this paper for publication we have been dismayed to learn that we had overlooked an important paper on the same subject (Zur Anatomie der Didymozoen by T. Odhner which appeared in a volume dedicated to Prof. Tullberg, Zoologiska Studier, Uppsala, 1907).

Odhner describes *Didymozoon scombri* and *Wedlia bipartita* and proposes to separate as *Didymozoon* those forms with separate sexes while for the hermaphrodite *D. bipartitum* he retains the old name *Wedlia* given by Cobbold. Although dealing with species different from those which form the subject of this paper he has described in detail most of the internal organs which we thought we were studying for the first time.

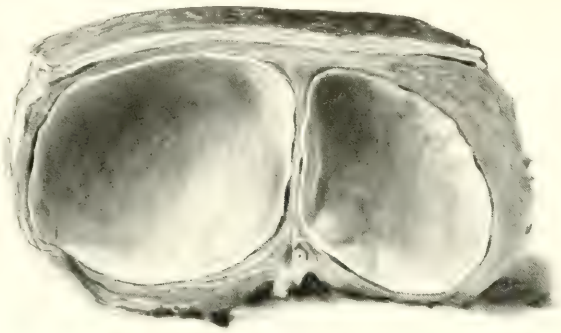
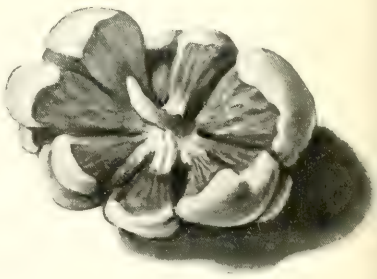


Fig. 1.



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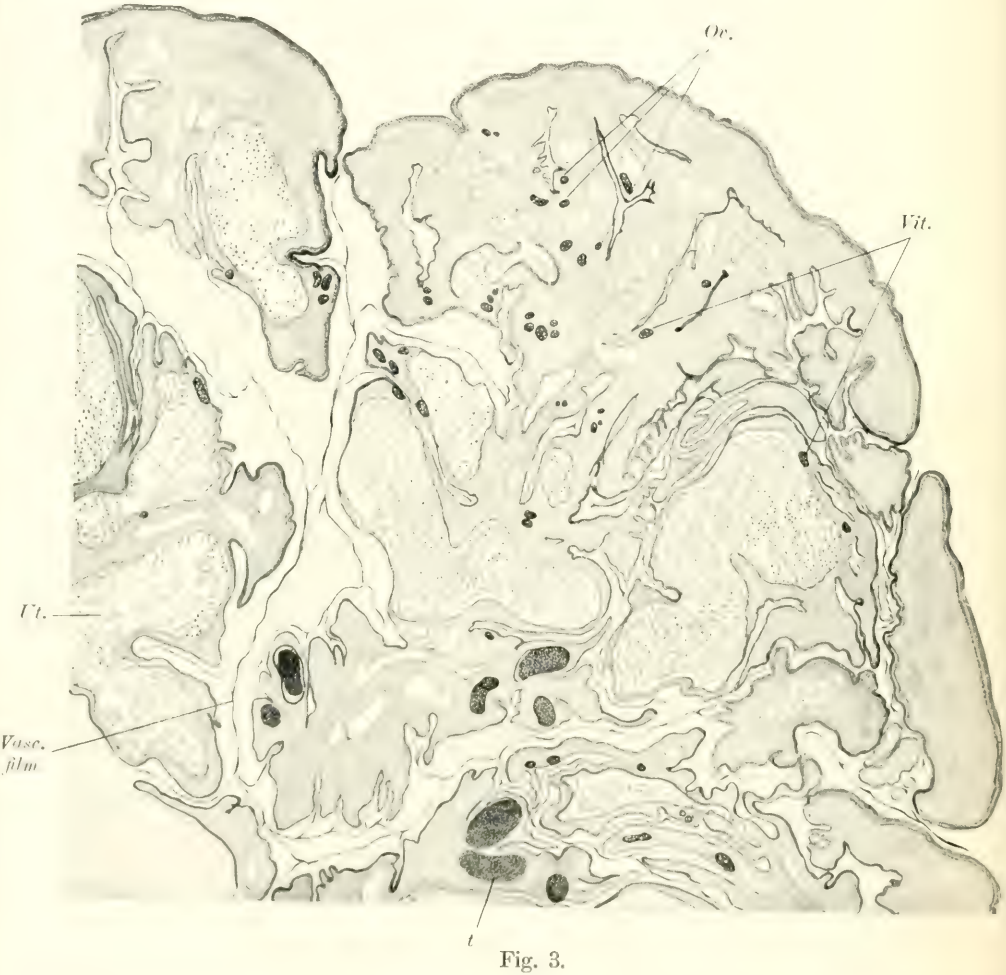


Fig. 3.

Fig. 4.

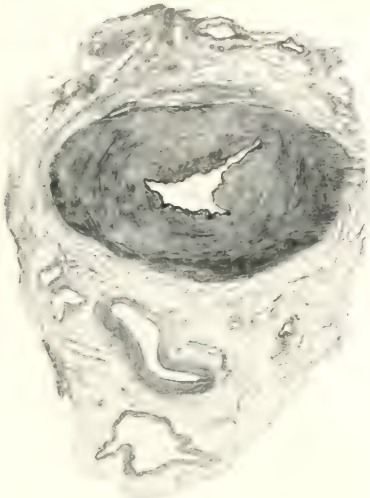
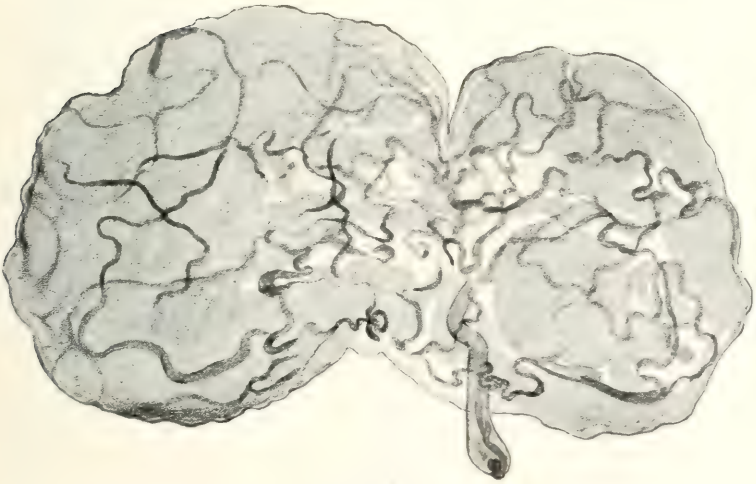


Fig. 5.



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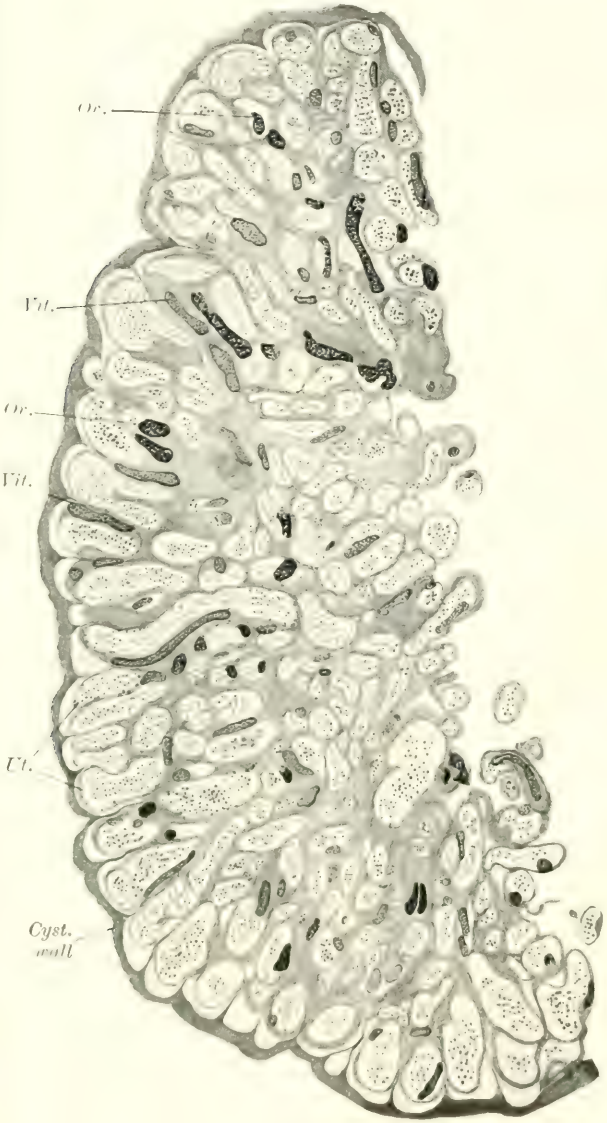

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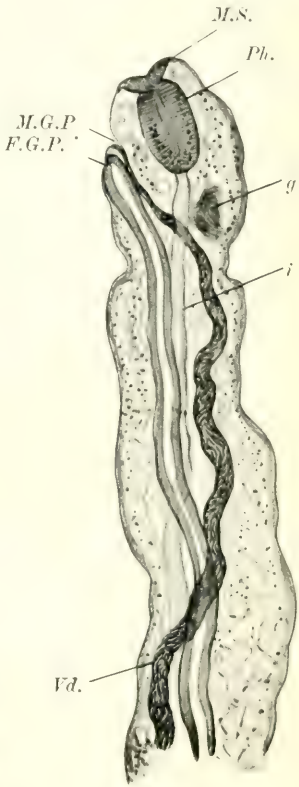

Fig. 8.

Fig. 9.

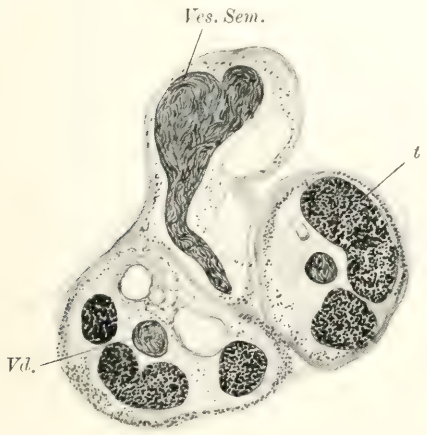

Fig. 10.

Fig. 11.

ıstav Fischer in Jena.

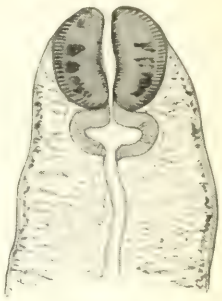

Fig. 13.

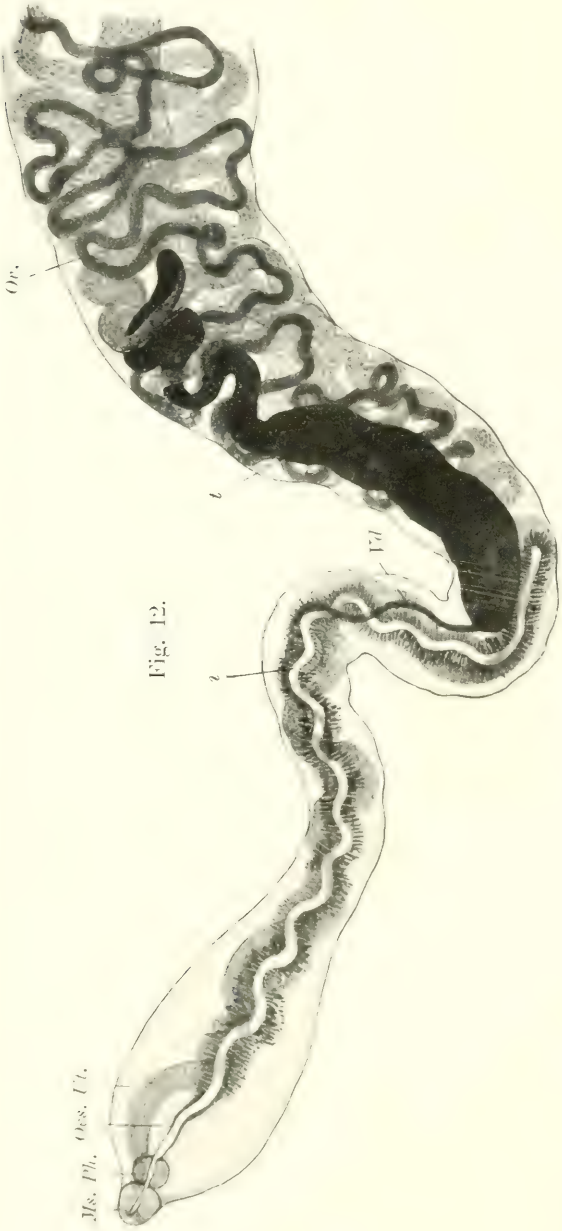

Fig. 12.

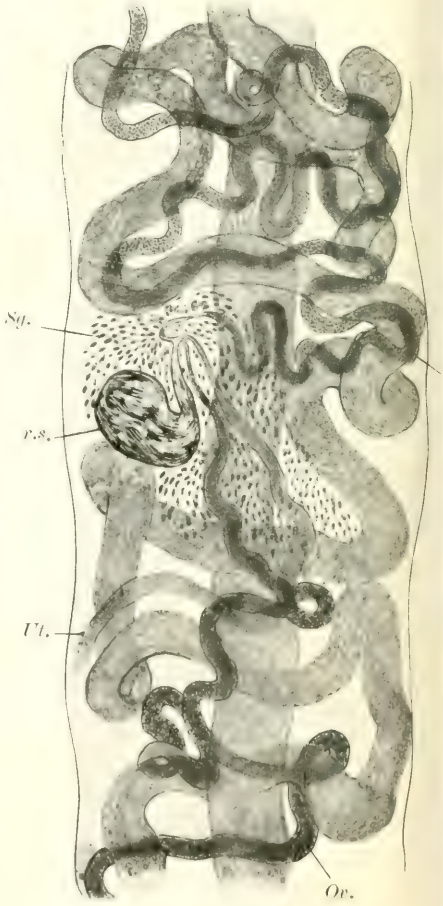

Fig. 16.

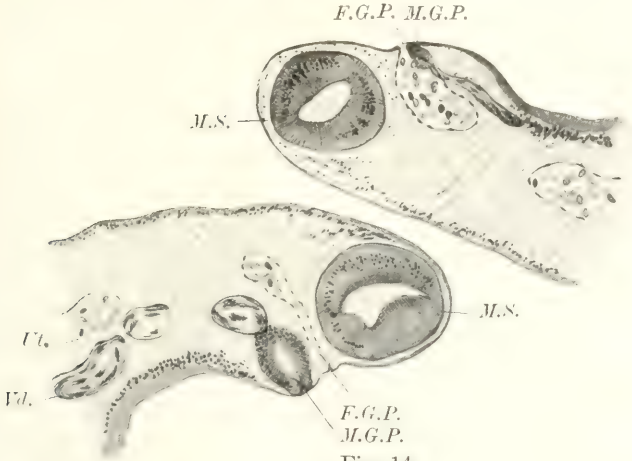

Fig. 14.

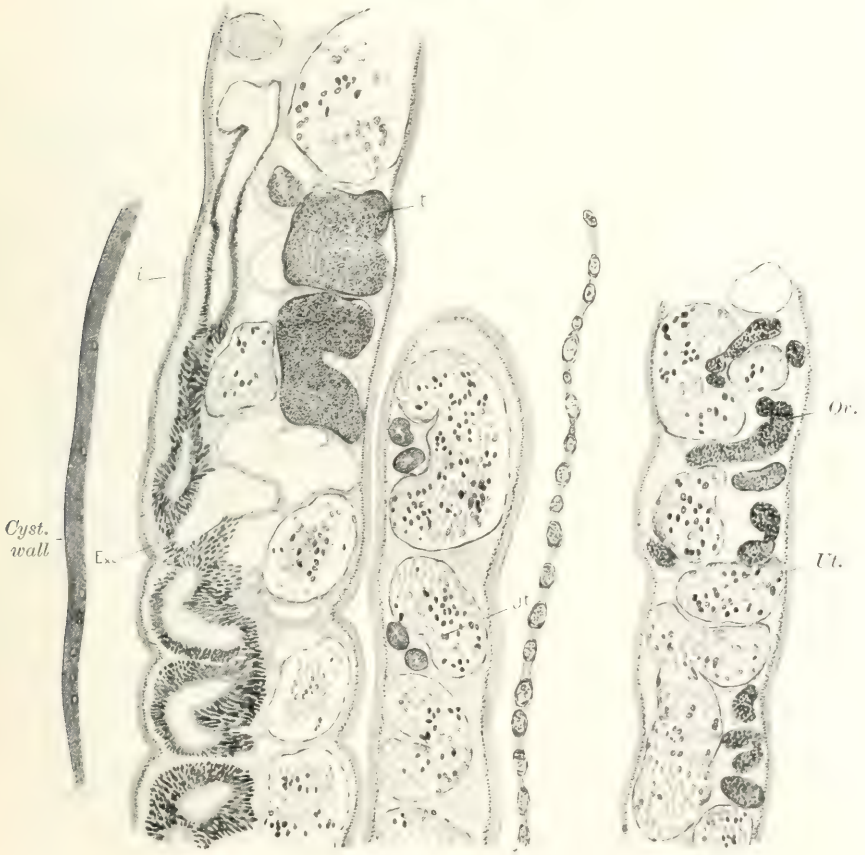

Fig. 15. *Vasc. film*

Each author is responsible for the scientific accuracy
and the proof reading of his contribution.

ACKNOWLEDGMENTS

The material for this work was received chiefly from the New York Aquarium and the Zoological Park of New York. My sincere thanks are therefore due to Dr. Townsend and Dr. Hornaday, Directors of these institutions, for all the privileges and courtesies shown me. Mr. Crandall, too, has been very kind and has assisted much in furnishing me with material from the Park.

Thanks are also due to Dr. W. G. MacCallum for several unique and new forms collected by him during his trip to the Orient.

Mr. A. Feinberg, artist, is also deserving of my sincere thanks for his patience and skill in preparing the plates.

FIGURES

ABBREVIATIONS

Abbreviations in explanation of plates:

a.—anus
b.c.—buccal sucker
b.—bursa
b.e.j.—bulbus ejaculatorius
c.s.—caudal sucker
c.s.—cirrus sac
c.p.—cirrus pouch
cl.—cloaca or genital pore
ce.—coeca.
cor.—coronet
c.—cirrus
c.gl.—cement glands
d.ej.—Ductus ejaculatorius
e.—eye
ex.p.—excretory pore
ex.v.—excretory vessels
g.i.c.—genito intestinal canal
g.p.—genital pore
h.—hook.
i.—intestine
li.—ligament
le.—lemnisci
l.c.—Laurers canal
m.—mouth
m.b.—muscular bands
m.g.—mucus glands
met.—metraterm
o.s.—oral sucker
ov.—ovary
oot.—ootype
oe.—oesophagus
ov.d.—oviduct
oes.g.—oesophageal gland
ph.—pharynx
p.p.—pars prostatica
pr.—prostate
p.—papillae post and preanal
res.—reservoir
s.g.—shell gland
s.v.—seminal vesicle or vesicula seminalis
s.r.—seminal reservoir
sp.—spicule
t.—testis
tr.—tridents
u.—uterus
u.g.—unicellular glands
v.d.—vas deferens
va.—vagina
vit.—vitellaria
vit.d.—vitelline duct
v.s.—ventral sucker or acetabulum
w.v.s.—water vascular system
y.d.—yolk duct
y.r.—yolk reservoir

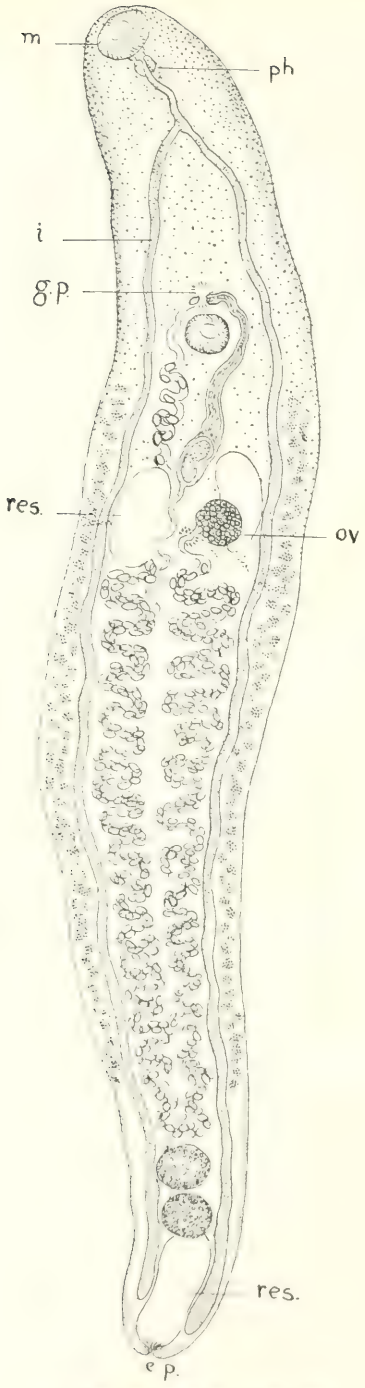

FIG. 87. *ILLORCHIS INSCULPTI*

NOTES ON THE GENUS TELORCHIS AND OTHER TREMATODES

Telorchis insculpti, sp. nov.

(Fig. 37)

Host—*Chelopus insculptus.*
Habitat—Intestines.
Locality—Zoological Park, New York. Eastern U. S.

Stunkard in the Journal of Parasitology, Urbana, Ill., December 15, Vol. 2, pp. 57-66 in his paper on the Trematode genus *Telorchis,* has very ably described the origin of the name by Luehe, 1889 (1899 Stiles and Hassall), and has given a fairly good general description of the anatomical structure of the genus with the variations noted in the various species. Perhaps in excessive detail, but nevertheless, such may be necessary on occasion. *T. insculpti* was found with others in the intestine of a wood turtle, *Chelopus insculptus* on January 4, 1918. It is a remarkably delicate formation, far more so than the plate shows. It is from 4 mm. to 5 mm. in length by .80 mm. in width.

It is wider at the head than at the posterior end, and for the anterior third of its length the skin is spiny, very much so at the head. In fact, the mouth is covered with spines both inside and without. The mouth sucker is 22μ wide and 12μ long. The mouth proper has its lips nearly touching and is only 12μ from side to side, while the anterior and posterior lips are only 7μ apart, and spines may be seen far back in the mouth. At the junction of the mouth sucker with the pharynx is a square portion which intervenes between these two structures. This is unusual and prevents the presence of a prepharyngeal oesophagus. It is 3μ from before backwards and is 10μ from side to side, is wider than the pharynx and shows at each end an apparent coecum or recess. The pharynx is 10μ wide and 8μ deep with the mouth lobulated. The oesophagus is 15μ long before bifurcation. From the bifurcation of the coeca to genital pore is 45μ. The pore is very spiny about its margin. The intestines are very narrow throughout. The acetabulum is 15μ in diameter. The cirrus sac is long, being 120μ and 15μ wide. There is on each side of the ovary a comparatively large water vascular reservoir, oval in shape, one 60μ long and 15μ wide, and the other 40μ and 20μ. At the posterior end, behind the testis, is another reservoir which is 45μ and 25μ. The ovary is round, situated about the middle of the worm at the end of the cirrus sac. It is 25μ in diameter. The testes are 25μ in diameter, more or less

circular in shape. The vitellaria extend from the genital pore on each side to within 40μ of the anterior margin of the anterior testis. It is arranged in delicate clumps in somewhat the shape of an expanded hand, but not properly shown in the plate. The uterus is in two columns, quite apart throughout, but they sometimes overlap the coeca. These latter extend almost to the posterior end. Eggs, 3μ wide and 5μ long. On each side of the pharynx is a cluster of unicellular glands.

Telorchis pallidus, sp. nov.

(Fig. 38)

Host—*Chelopus insculptus.*
Habitat—Intestine.
Locality—New York Aquarium.

This worm was found on January 4, 1917, in a wood turtle along with some others of the same family, two good specimens being recovered. It is, however, so different from the others in structure, that it must be recorded as a new species. It is comparatively long, unarmed with spines on its skin, and very slender, being 7 mm. long and 55μ in width, and it is almost diaphanous, so delicate is it in structure. Its anatomy is somewhat different also. The mouth sucker is subterminal and rather oblong, being 20μ long and 15μ wide. The pharynx, 10μ long and 10μ wide, prepharyngeal oesophagus 10μ long, genital pore from division of coeca is 100μ. Length of cirrus and cirrus sac 135μ long, very narrow and extends almost to the ovary, which is situated about the middle of the body, both as regards the length and width. The ovary is round; and the genital junction is very distinct, a short distance posterior and to the left. This structure is not any different from usual, the oviduct is given off about the middle of the left side and proceeds backwards, and receives a delicate vitelline duct from each side. The ootype and shell gland are comparatively large. The seminal reservoir is situated on the right side of the genital junction. The uterus, full of eggs, extends from here posteriorly nearly to the anterior testis, and is bounded on each side by the intestines. These latter terminate very near the posterior end of the worm in quite widened coeca, and enclose or overlay a fairly large reservoir of the water vascular system. The testes are very small, being but a little larger than the ovary. The posterior one is 60μ from the end of the worm and the anterior one is 20μ in advance of its mate. The vitellaria are exceedingly sparse, being just a few small glands on each side, chiefly between a point opposite the ovary and 100μ further posteriorly. The glands are small and much scattered. There are two oval reservoirs, one on each side opposite the middle of the cirrus sac, the one the right being about twice the size of the left one.

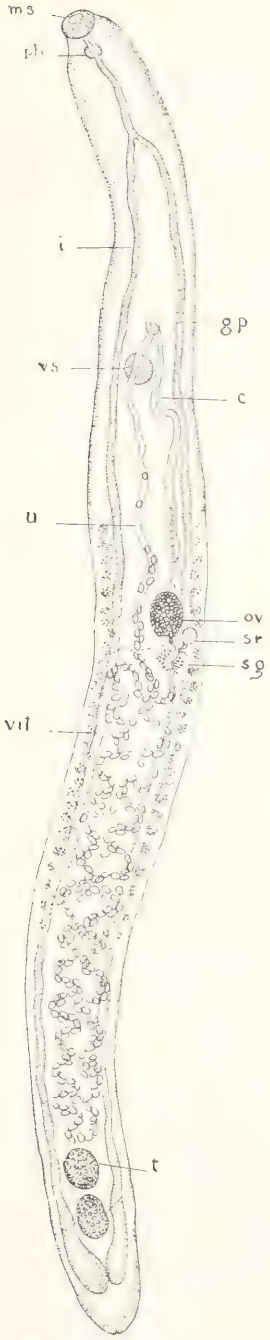

FIG. 38. *HELORCHIS PALLIDUS.*

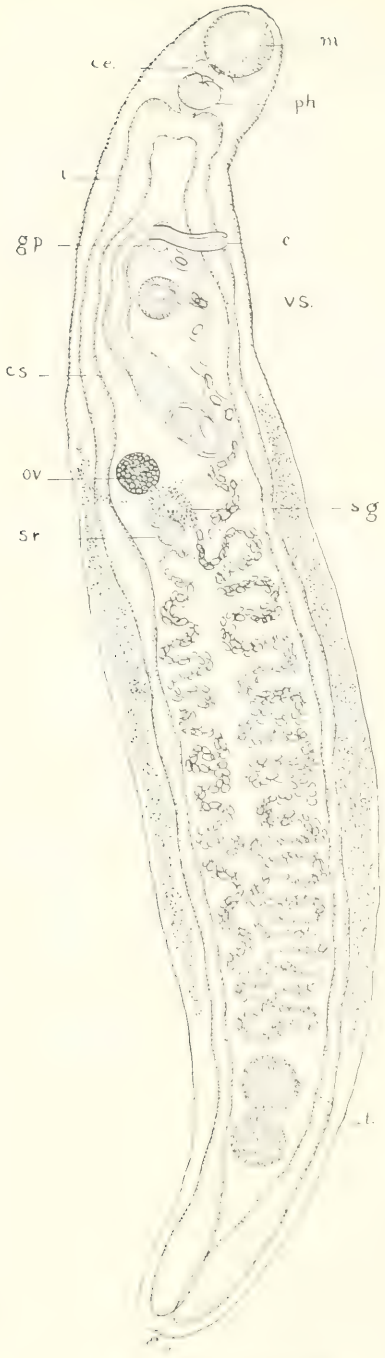

FIG. 80. *HETOPCHIS CHILOPS*

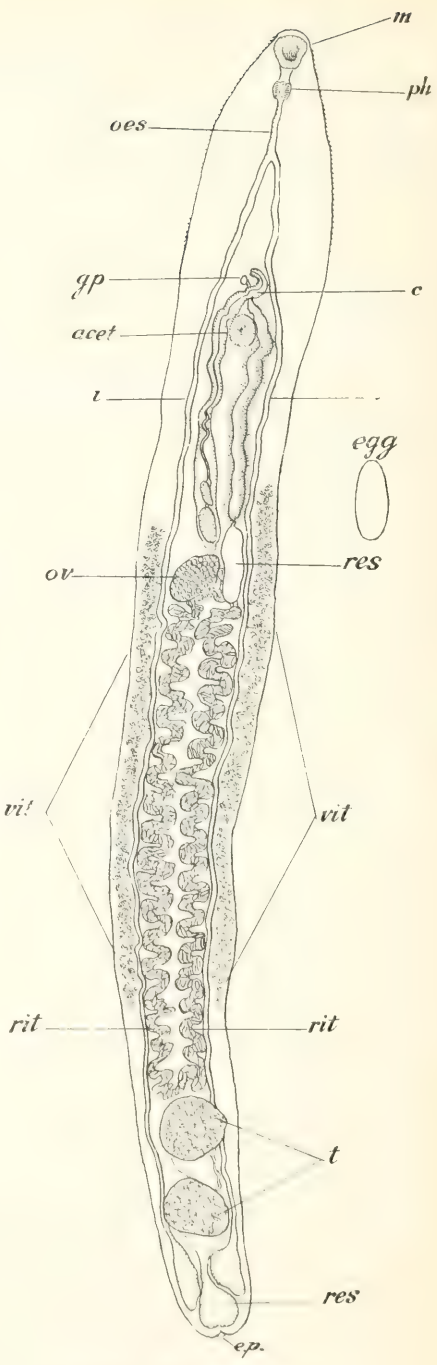

FIG. 81. *HETORCHIS GUTTATUS.*

Telorchis chelopi, sp. nov.

(Fig. 39)

Host—*Chelopus insculptus.*
Habitat—Intestines.
Locality—New York Aquarium.

This worm was found with a number of others in the intestine of a wood turtle, *Chelopus insculptus* on January 4, 1918. It is a large coarse looking worm compared with some others of the same genus. The mouth sucker is 45μ across and 40μ deep. The anterior edge of the sucker is cut off square or deeply notched, and the posterior margin is separated from the pharynx by an oblong structure 25μ from side to side and about 5μ from before backwards. The outer ends of this structure seems to have a coecum at each end, and outside on each side is a mass of deeply staining unicellular glands. There is consequently no prepharyngeal oesophagus. The pharynx is roundish except in front, where it is lobulated. It measures 20μ in diameter. It is 50μ from the pharynx to the genital pore, that is from the division of the coeca. The oesophagus is very short, not more than 10μ, and the coeca are large and lobulated and on each side the angle returns towards the head, nearly to the pharynx, after that they proceed on each side in wide lobulated curves to the extreme end of the worm. Cirrus sac, 180μ long and 35μ wide. Ovary round, 25μ across. The amount of vitellaria is little compared with other forms. The glands extend 160μ on each side. Testes, 30μ in diameter. The intestines are quite clubbed at the posterior end. The cirrus is large and generally exerted.

Telorchis guttati, sp. nov.

(Fig. 40)

Host—*Chelopus guttatus.*
Habitat—Intestine.
Locality—Zoological Park, New York.

This worm is a mature female full of eggs and is 8 mm. long and 1 mm. wide. It was found on January 4, 1918, in the intestine of a spotted turtle, *Chelopus guttatus*, and is a fine specimen of a Telorchis. It is pointed at both ends. At the anterior end, the mouth sucker is somewhat conical in shape, being narrower posteriorly than in front. It is 15μ long and at its widest part also 15μ, with the mouth proper in the centre. It has a very short prepharyngeal oesophagus this being about 5μ long. The pharynx is 8μ long and 10μ wide, lobulated entrance. Oesophagus, narrow and 35μ to the division, and 90μ from

there to the genital pore. Anterior third of the body, spiny. Cirrus sac 200μ long. Acetabulum about the same size as the pharynx. The metraterm 101μ and studded on its outside by numerous unicellular glands. Ovary, 25μ in diameter and situated at the end of the cirrus sac. Vitelline ducts plainly visible, Laurer's canal and the seminal reservoir not seen. Eggs 5μ long and 3μ wide. Vitellaria delicate and extending from ovary in front to near testis behind. Uterus fills up the body cavity between the intestines and in places overlaps them. To the left of the cirrus sac is a water vascular reservoir 30μ long and 10μ wide. It is situated dorsal to the cirrus sac. The testes are two, very near the posterior end and situated between the ends of the intestines, where these latter are much lobulated. The vitellaria are very plentiful, they extend from a point some distance in front of the ovary to near the anterior testis posteriorly, and the glands are in clusters. The posterior reservoir is 100μ long and dorsal to the testes. This worm is much like No. 2 as shown in Stunkard's worms, only it is larger. In fact, it is difficult to compare these worms for some writers must measure immature forms. An average size of the mature specimens would render the diagnosis much easier.

Distomum auritus, sp. nov.

(Fig. 41)

Host—*Aplodinotus grunniens.*
Habitat—Small intestine.
Locality—New York Aquarium. Nearby fresh-waters.

During the examination of a fresh-water drum, *Aplodinotus grunniens* Rafinesque, 1818, June 26, 1916, kindly sent me from the New York Aquarium, there were found in the intestine a lot of distomes. The fish had died of a severe attack of myxosporidia, and as usual, when a fish is in a weakened condition, that state seems to encourage the infestation of its parasites. The same rule holds good in the animal, as well as in the vegetable kingdoms. In this case the gills were found to harbor *Microcotyle* and the intestines had in them a number of very lively distomes, which I have ventured to name according to a very prominent external feature. The skin about the head is redundant, and the ear-like appendages are merely rolls of this which keep in the forms of ears. Although the host had been dead for a few hours, the worms were still very much alive, extending themselves and herding and clinging together. The mouth sucker is large, subterminal, and circular, and immediately posterior to it is a fairly large pharynx, followed by a curving oesophagus. This organ is long and divides at the junction of the anterior fifth with the posterior four-

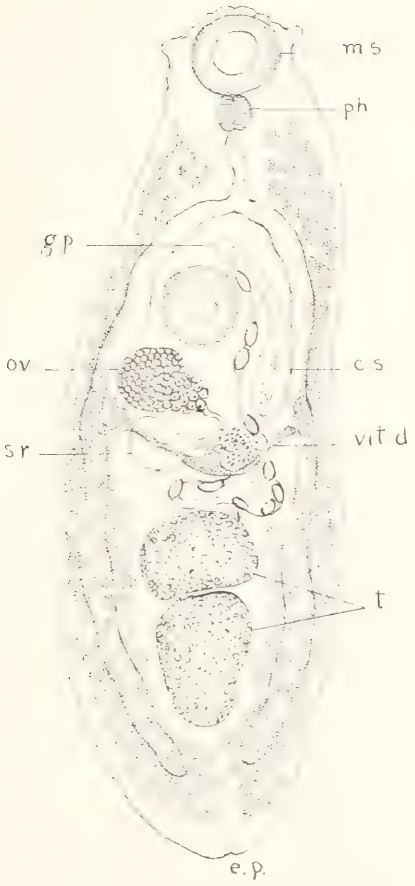

FIG. 31 *DISTOMUM AURITUS.*

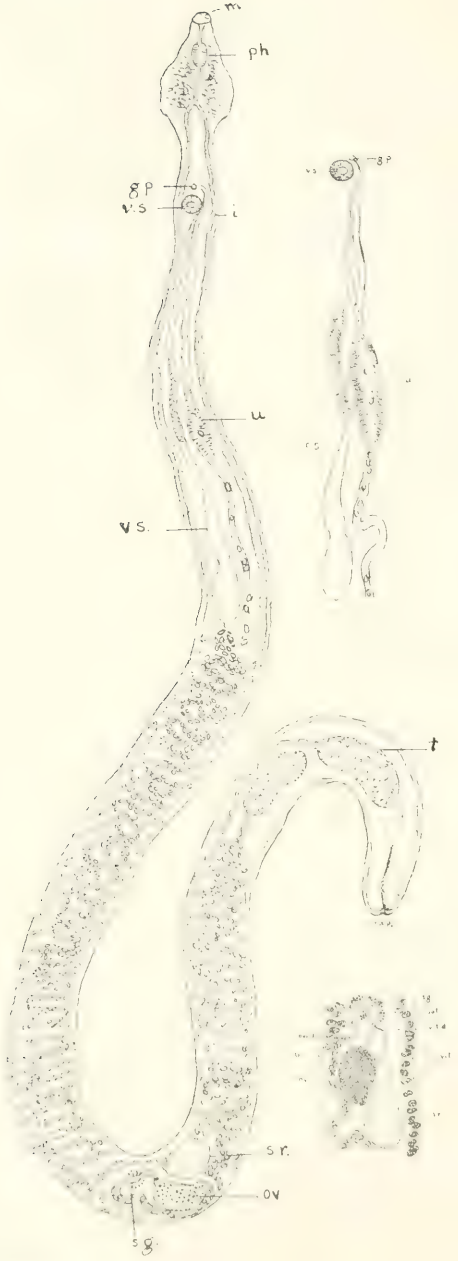

FIG. 42. — *DISTOMUM SPECULIFERUM*. FIG. 42 A. *MELICERTERM* AND *DUCTUS EJACULATORIUS*. FIG. 42 B. GENITAL SCHEME.

fifths of the body length. The coeca are relatively large and extend to nearly the posterior end of the body. The acetabulum is situated one-third of the length from the head, and a short distance anterior to this is the genital pore. Almost immediately behind it on the right side is the ovary, the oviduct from which first proceeds backward to join the vitelline ducts, and the duct from the seminal reservoir. The ootype and shell gland are also here in the centre of the body, but Laurer's canal and the ductus genito-intestinalis were not seen. The testes are two, one rather cubical in form and the posterior one conical. The vas deferens enters into a good-sized cirrus sac. Eggs, large but not numerous. The vitellaria, very profuse. Skin, smooth.

For the purposes of classification, this, which averages in length 3 mm. and .40 mm. in width, probably belongs to the family *Allocreadium Lss,* with *A. isoporum* as the type; although the description does not quite correspond, nor does it correspond with the description of *Isoporum armatum* of W. G. MacCallum.

Distomum spiculiferum, sp. nov.

(Figs. 42, 42a, 42b)

Host—*Acipenser sturio.*
Habitat—Intestines, spiral valve.
Locality—New York Aquarium.

This peculiar worm was found in the spiral valve of an *Acipenser sturio.* There were twelve or fifteen of them. The host died at the New York Aquarium and was a very old and large specimen, weighing probably about eighty pounds.

The worm is quite large and has a spear or arrow-head-shaped head, and this on the surface shows numerous canals, probably part of the water vascular system, and since they are stained with carmine they look exactly like the capillary circulation in an inflamed part of an animal. The head is 170μ across and 200μ long, and quite snake-like in appearance. The mouth is terminal and has a buccal sucker on each side. It is 15μ in diameter. The prepharyngeal oesophagus is short, about 10μ long. The pharynx is 20μ long by 10μ wide, and the post-pharyngeal oesophagus is 30μ long before it divides into the coeca, and the genital pore is about 100μ posterior and just anterior to the ventral sucker or acetabulum, which is relatively small, being 25μ in diameter. The worms average 22 mm. in length by 17.50μ in width at the widest part. The most peculiar feature is the character of a portion of the interior of the vesicula seminalis and also a portion of the interior of the uterus, as these organs approach the end of their course. The vesicula seminalis is large at the posterior end and very long, terminating

in the cirrus, and the canal is studded throughout its lumen with rather large spicules which are thick at the base and from 5μ to 8μ long. They extend throughout a distance of 180μ, after which the prostatic muscular portion of the cirrus is as usual. The uterus also presents this peculiarity throughout a distance of 210μ, and here the organ is twice as wide as elsewhere. It is fusiform in this locality. The spicules are about the same size as those in the vesicula seminalis, but seem more plentiful, are apparently tangled up some, and some of them are loose. Many eggs are seen in the uterus at this part at the same time. The eggs are 4μ long by 2.50μ wide, are yellow and oval. The uterus fills the greater part of the body and is full of eggs.

The ovary is situated at about the junction of the posterior and middle thirds of the body, where is also plainly seen the genital junction, the seminal reservoir, and the shell gland. The ovary is 40μ long and 30μ wide. The testes are two oblong bodies very near the posterior end. They send two separate vasa efferentia forward throughout half the length of the worm to join the vesicula seminalis. The testes are 230μ long by 70μ wide. The intestines extend quite to the posterior end and there between them is the excretory pore. The vitellaria are situated on each side as far as the body of the uterus extends.

Eurema keksooni, gen. et. sp. nov.

(Fig. 43)

Host—Small Ray.
Habitat—Gills.
Locality—Singapore.

On July 20, 1916, there was found on the gills of a small unspotted ray a small worm which is unusual. It was found at Singapore by Lee Kek Soon, who was assisting Dr. W. G. MacCallum. He said it was from the gills, but as a matter of fact, it was found at the bottom of the dish after washing the gills and may have come from the gullet or mouth. He was confident, however, that it came from the gills. The spiral valve of the host yielded several cestodes of different species.

The anatomy of this worm is so unusual that it could not escape being classed as a new genus and species. The mouth is an unusually wide, loose, open structure with, apparently, the pharynx opening at once between the lips, or immediately behind them at the middle of the wide mouth. The head is much wider than the neck, and the lips extend the entire width of the head. The neck is short and the body begins to enlarge at once. At the shoulders, as it were, or near the widest part of the body is seen on each side a distinct prominence, like that in some of the *Polystoma*. On close inspection, these two

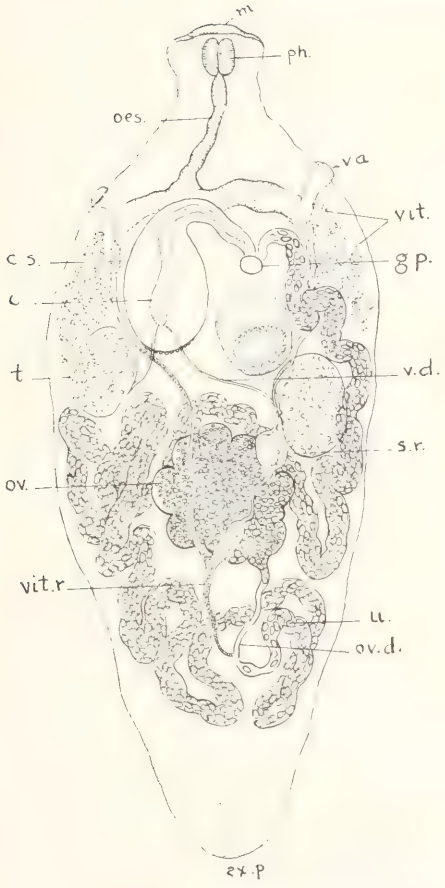

FIG. 13. *EUREMA KEKSOONI.*

proved to be the entrances, for there are many, to the vagina. This feature in itself is peculiar, for the worm has not any of the other features of a polystome. Between these two structures and slightly posterior to them, yet in the middle of the body, is the acetabulum, of good size. The vaginae both lead backward and inwardly, one to join the large and widely-spreading right hand main duct of the vitelline reservoir, which proceeds superficially over and ventrally to the large ovary, to a point considerably posterior to that organ, where it helps to form the genital junction, partly under massive folds of the egg-filled uterus. The other passes through a seminal reservoir and on to join the left branch of the vitelline reservoir. The pharynx is an unusually large structure for so small a worm, and it is exceedingly muscular. There is no prepharyngeal oesophagus, but the oesophagus posterior to the pharynx is large and fairly long before it divides into the ceca. These are voluminous and extend nearly to the end of the body. The ceca enclose the genital pore in front of the acetabulum. This is overhung by a relatively large muscular cirrus, which is partly enclosed in a wide cirrus sac, the posterior end of which is covered with a number of round, cell-shaped pedicles or papillae. The ovary is a large lobulated structure situated in the centre of the body, and posterior to the acetabulum. Anterior to it and to the left is seen the seminal reservoir, with the vagina, on that side, entering its base. The vagina on the other side may, and probably does, enter the right arm of the vitelline reservoir. The uterus is large and its folds fill the posterior part of the body and as far forward as the acetabulum. The water vascular system is plainly visible on each side of the neck, in the form of three branching tubules. At the posterior end of the worm, at its centre is the excretory pore, with small tubes concentrating to it from the fairly wide posterior end. The skin is smooth, thick, and unarmed. The eggs are oval and yellow.

Measurements of Eurema keksooni

Length	3.00 mm.
Width	1.20 mm.
Head width	.40 mm.
Pharynx width	15μ
Pharynx length	20μ
Eggs length	2μ

Paramphistomum chelydrae, sp. nov.

Family—*Paramphistomidae* Fisch.
Genus—*Paramphistomum* Fisch.

(Fig. 44)

Host—*Chelydra serpentina.*
Habitat—Rectum.
Locality—New York Aquarium.

The worms which form the basis of this short paper were found on March 17, 1915. There were three of them in the rectum, all mature, containing eggs, were all of the same size, and I believe adult specimens. They were 5.50 mm. long and 1.60 mm. wide. General outline of the body oblong, narrow at the mouth, becoming wider until about the middle, where it reaches its greatest width. Then this gradually lessens until nearly opposite the middle of the disc, when it suddenly ceases and ends in the middle of the side of the disc, thus forming an angle, which, as far as I know, has been unusual in this genus. The disc, thus is shown to extend half of its length past the end of the body.

The plate given here is as accurate as a toto mount could be made. No oral evaginations or coeca can be seen in the mouth sucker of two of the worms, but in the third they can be seen, and it is to be presumed that they are also present in the others, for this is very common in the *Paramphistomidae.* The mouth sucker is terminal and bowl-shaped, 80μ long and 60μ wide. Opening of the mouth, which is terminal and corrugated longitudinally is 25μ from side to side. Oesophagus, including the oesophageal bulb or pharynx, 80μ long. The bulb or pharynx is situated just anterior to the division of the intestinal coeca, and is 25μ long and 25μ wide. The intestines are very irregular in shape and extend to within a short distance of the posterior sucker. The genital pore is central and placed in the angle of the coeca. The ovary is perfectly round or globular, and is 30μ in diameter. It is situated a considerable distance posterior to the testes. The genital junction can be fairly well defined posterior to the ovary. The uterus is a comparatively short tube, and generally contains three eggs which are 20μ long and 12μ wide, oval and yellow. Testes are placed well towards the anterior part of the body cavity. They are irregular in shape, indeed quite branched or clubbed, one 43μ long and 25μ wide, and the other 50μ long and 40μ wide, one in advance of the other, and in all three specimens, the internal organs are very much alike. Vitellaria plentiful, extending from near the genital pore on each side to near the end of the intestines posteriorly, and all across the body in large masses. The disc is rounded or rather oval, the central opening being cordate in shape, with the small end posterior. The two angular portions of the body are situated opposite the middle of the margin of the disc, and extend in a line across the middle of

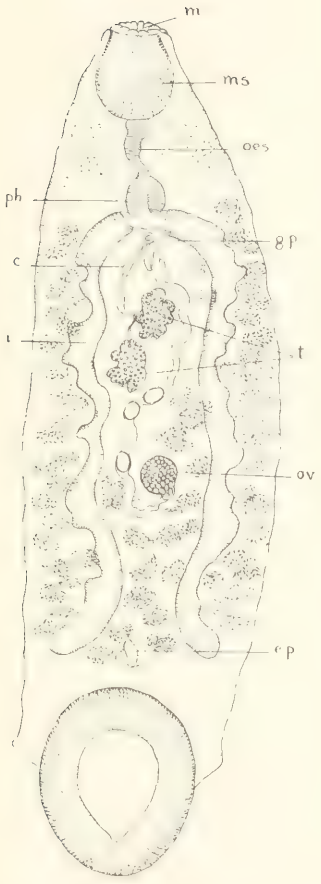

FIG. H. *PARAMPHISTOMUM CHELYDRAE*

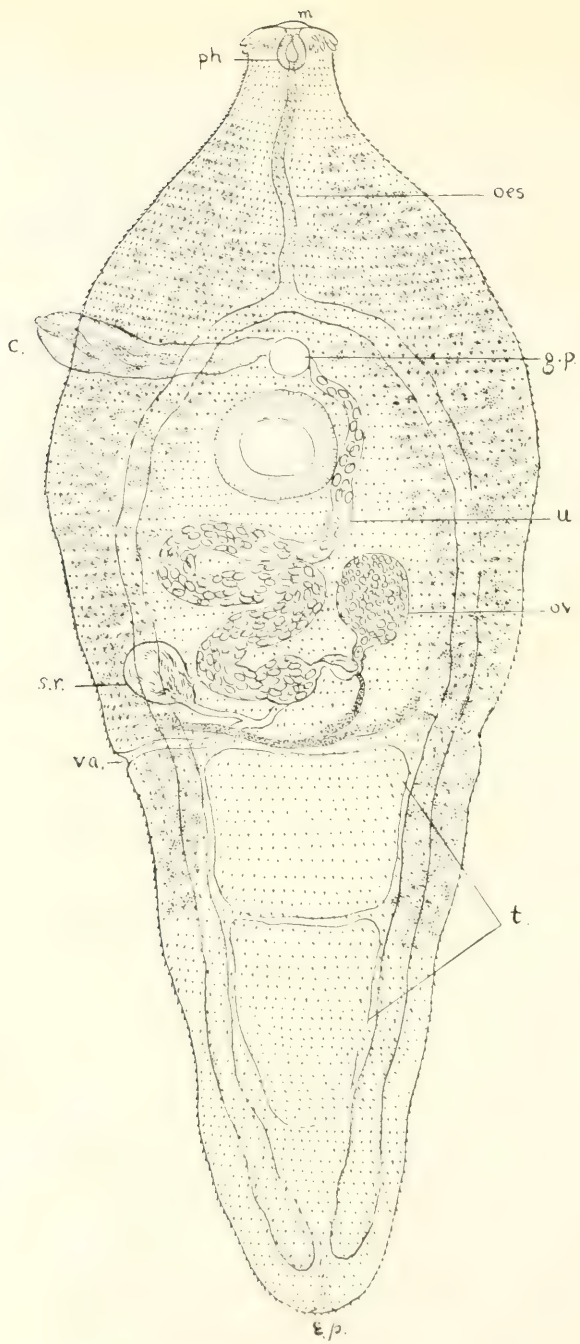

FIG. 35. *ECHINOSTOMUM HERODIAE*

the disc dorsally, so that the posterior half of the disc projects beyond the body of the worm. It is 130μ anteroposterior and 115μ across, and internally 75μ and 45μ. The excretory pore is large and situated just anterior to the disc in the middle of the body.

An odd thing about these particular worms is that they were all infested with great numbers of small yellow cysts, which may or may not have been calcified before death, however, they now appear throughout the parenchyma and over the surface of the organs as small round bodies.

Echinostomum herodiae, sp. nov.

(Fig. 45)

Host—*Herodias timoriensis*.
Habitat—Gall bladder and ducts of aquatic birds.
Locality—Java.

This worm was found by Dr. W. G. MacCallum in the gall bladder of *Herodias timoriensis* in Singaradja Bali. Several were found and from its exceedingly spiny skin, it must have been a source of great irritation to its host. It is spear-shaped and the head shows the mouth terminal and surrounded by several long papillae. The anterior portion of the head is unarmed, but the skin throughout over the body is covered thickly with prominent chitinous spines arranged in transverse rows across the body so thickly indeed that they overlap somewhat. The pharynx is very close to the mouth and is 15μ long by 10μ wide. The oesophagus is long and tortuous, is divided at the junction of the anterior and second-fifth of the length of the body. The coeca spread widely and enclose a large genital pore, from which the cirrus is generally seen exserted. This is large and club-shaped, and measures 100μ in length. The genital pore is at the anterior margin of the acetabulum, and this latter organ is 100μ in diameter. The ovary is irregular in shape, is about 50μ in diameter, and is situated almost directly behind the acetabulum. Wide vitelline ducts meet the oviduct here from each side, as does also the duct from the seminal reservoir. This sac is to the left of the ovary. Laurer's canal can not be made out with certainty on account of the presence of numerous large eggs, which are 7μ long by 4μ wide. The testes of which there are two, are large and are of different size and shape. The anterior one is irregularly square, filling largely the abdominal cavity from side to side. It measures 1.40 mm. across, and 80 mm. from before backward. The other is more or less conical to fit the posterior part of the body cavity, and is about 1 mm. long by .60 mm. wide. The intestines extend to the posterior end of the body and are there club-shaped. A large excretory pore opens at the posterior end and one

can see a system of ducts coming from the body. This worm looks somewhat like that described by Ramsay Wright as having been found in Botaurus minor North America, and named by him E. asperum.

Measurements of E. herodiae

Length	8 mm.
Width	3 mm.
Acetabulum	100μ in diameter
Head across	45μ

Axine carangis, sp. nov.

(Fig. 46)

Host—*Caranx hippos.*
Habitat—Gills.
Locality—New York Aquarium. Common on East coast.

The name *Axine* was first given to this genus by Abilgaard in 1794, but this particular species appears to be new.

This worm was found in great numbers on the gills of a *Caranx hippos* after they had caused the death of their host. It is a very graceful creature, perhaps the most delicately shaped of all the *Microcotyle.* The head begins with a small protuberance, presumably the mouth or lip and on each side of this and immediately behind it, is the mouth proper with a buccal sucker on each side. A short distance posterior to this is the genital pore, which is divided into two portions of a circle, each being surrounded with a row of spines or hooks, and a very short distance posterior to this appears the armed head of the cirrus, and still further posterior in the middle of a more or less clear circular space is the opening of the vagina which is also armed with very fine spicules. This opens on the dorsal surface, as in other members of the microcotyle family.

The uterus is usually filled with a column of fusiform eggs making their way to the genital pore where they are ejected or laid. At either pole of the egg is a long filiform filament which, when the egg is laid, becomes attached to the filaments of the gills, where it is retained until hatched. The ovary which is an irregularly-shaped mass of germ cells enclosed in a roughly horseshoe-shaped sac, lies about the middle of the worm. The vitelline reservoir, which receives the spermatozoa from the vagina, lies in a somewhat V-shaped structure and adds its share to the oviduct in the formation of the egg. The seminal reservoir is always plainly to be seen in this locality, and behind this genital junction are situated the testes, about forty or fifty in number. The vitellaria are very profuse and extend from near the

FIG. 46. AXINE CARANGIS.

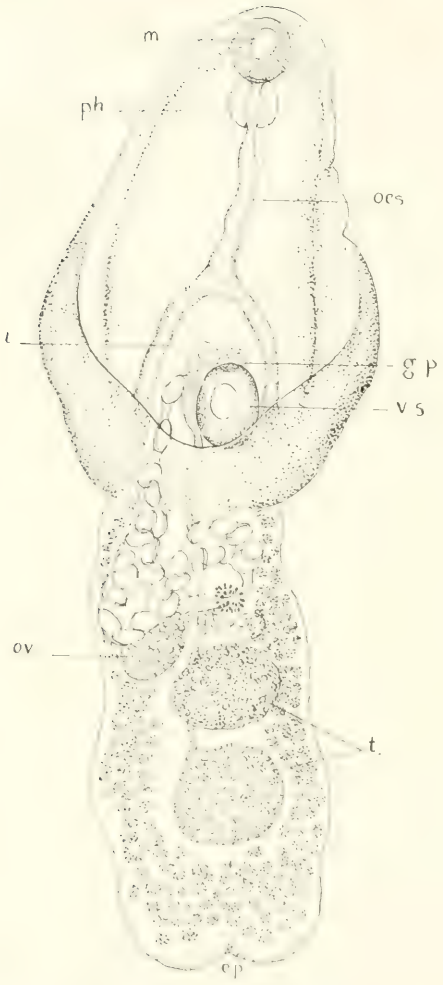

FIG. 3. — *HEMISTOMUM HETEROSTRUM*

head on both sides of the worm into the disc at the posterior end. This latter structure is very graceful. It has a single row of claspers along the margin. They seem to be of about the same shape as those of the *Microcotyle,* but are more delicate. The disc is the widest portion of the worm, being about 1 mm. in width and shows about 90 suckers along its margin. The body of the worm at its widest is only about .80 mm., and it is only 7 mm. long.

They seem to be very productive, for like the *Microcotyle,* they often cause the death of the fish they infest from sheer loss of blood probably plus the poison which all of these worms are said to impart to their host.

The fish was kindly sent for examination by the Director of the Aquarium.

Hemistomum haustrum, sp. nov.

(Fig. 47)

Family—*Holostomidae.*
Genus—*Hemistomum,* Diesing.

Host—*Alutera schoepfii.*
Habitat—Intestines.
Locality—Southeast coast of the United States.

On May 4, 1915, a large, but very thin *Alutera schoepfii* was kindly sent me from the New York Aquarium. On examination, it was easy to see why this host was emaciated. On the gills was a good-sized Branchiobdella about 1½ inches in length. In the urinary bladder were a lot (46) of *Catoptroides aluterae.* On the gills there were a great many small copepods, and in the intestine a number of small distoms of two or three species, also a lot of *Hemistominae,* which I am about to describe. There were forty or fifty of this species recovered, which are so very unusual in form. It is roughly somewhat the shape of a scoop, such as is used by grocers, the tip being the head where appears the subterminal mouth sucker followed without interval by the pharynx. The oesophagus is long and divides into the coeca about the middle of the dilated portion of the anterior half of the worm. In the somewhat triangular space enclosed is the acetabulum, and just anterior to this the genital pore. At this point too may be seen the adhesive disc which covers in largely the acetabulum. The intestines appear to extend nearly to the posterior end of the worm on the dorsal surface of what might be called the handle of the scoop. The eggs in the uterus are also nearer the dorsal than the ventral surface. There are two good-sized rounded testes and in front of them to their right is the ovary, which is about half the size of a testis. The oviduct is given off from the left side and posterior to this tube is

the seminal reservoir, and here too is situated the genital junction of the vitelline tubes, oviduct, and ducts from the seminal reservoir, all near the ootype and shell gland. The other ducts supposed to be seen here, Laurer's canal and the ductus genito-intestinalis, so seldom seen by me without sectioning, are not to be seen here in a toto mount, even if they are there. The vitellaria are very plentiful throughout the posterior half of the body, and in fact, all of the organs seem confined to this portion except the outlet of the uterus and the cirrus with its sac. The vasa efferentia join to the form the vas deferens, which, as usual, ends in the cirrus sac.

Measurements of H. haustrum

Length	2.25 mm.
Width	1.00 mm.
Width of posterior part of body	.60 mm.
Egg oval	.05 mm.
Mouth sucker	.20 mm.
Pharynx length	.150mm.
Ovary	.40 mm.
Testis	.60 mm.

Spirorchis, gen. et. sp. nov.

(Fig. 48)

Host—*Chelopus insculptus.*
Habitat—Intestine.
Locality—New York Aquarium.

These three very unusual worms were on January 4, 1917, found in the intestine of a wood turtle. As will be seen by the color of the contents of its intestines, it is a haematophagic trematode. It is 4 mm. long by .80 mm. wide, with a smooth skin throughout. Its body tapers toward each end, being widest about its middle. The anterior end displays a mouth sucker, which is almost entirely external to the head, being joined thereto by a prepharyngeal oesophagus, 30μ in length. The postpharyngeal oesophagus being 25μ. The coeca begin at about 60μ from the mouth. The pharynx is not more than 5μ in diameter. Almost at once in the angle of the coeca, the testes begin by a conical mass, which is followed by nine other irregularly-shaped masses, all joined together in a rough spiral column filling almost the whole abdominal cavity between the intestinal coeca. They finally end in close touch with a large, more or less conical-shaped seminal reservoir, which in itself, is larger than any one of the testes. Its base is anterior, and it gradually tapers into what probably is the cirrus, although, on ac-

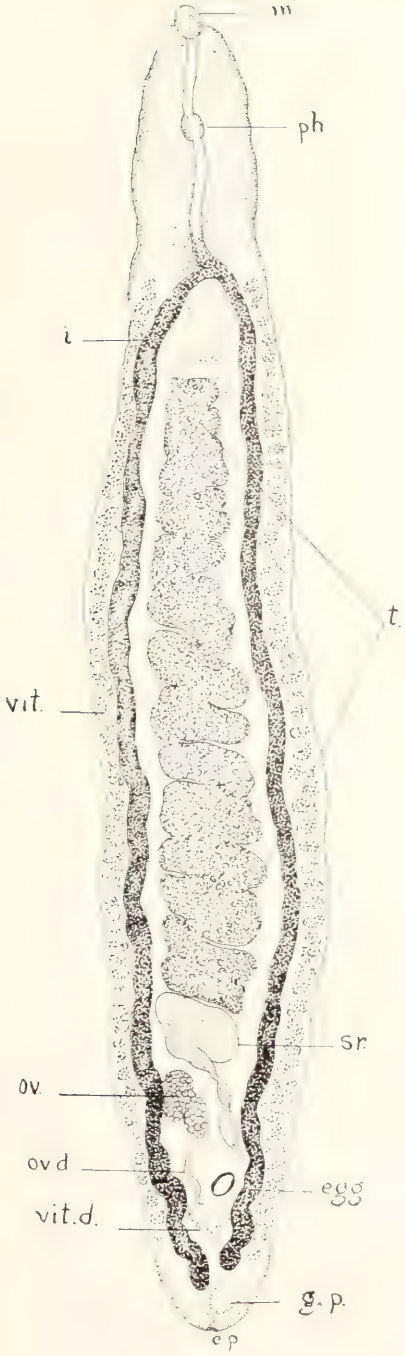

FIG. 48. *SPIRORCHIS*

FIG. 39. *ACANTHOBDELLA MICHELI*. FIG. 39 A, COROLLE. FIG. 39 B, EGG.

count of the black intestines filling the posterior end of the worm, it is not possible with the material at hand to trace the genital scheme. The vitellaria are very profuse, and the ordinary Y-shaped reservoir can be plainly seen. The ovary 20μ in diameter with a long oviduct directed posteriorly is seen near the end of the seminal reservoir, but no ootype or shell gland or other seminal reservoir can be seen, nor indeed, can the uterus be made out. One large, oval egg, 10μ long and 5μ wide, is seen posterior to the ovary in each specimen. The shell of this seems thick and cleavage is well advanced. The genital pore is a little posterior to the tips of the coeca and between them. It is more or less circular, but its connection with the oviduct or uterus cannot be seen, indeed, in one case the black coeca have coalesced and formed a large black mass over this interesting region. No acetabulum or other adhering organ seems to be present. The usual excretory pore is to be seen at the posterior tip of the body. On account of the unusual spiral mass of testes, filling the abdominal cavity to the exclusion of everything else, I have taken the liberty of naming the worm *Spirorchis* as a gen. et. sp. nov.

Acanthodiscus mirabile, gen. et. sp. nov.

(Fig. 49)

Host—*Caranx hippos.*
Habitat—Gills.
Locality—New York Aquarium.

The host is common on both the east and west coasts of the United States.

This very strange ectoparasite was found on April 18, 1917, in considerable numbers on the gills of a crevalle, and I have no doubt that at times the species may be a serious menace to the life of the fish.

One can hardly say how many were on the gills, for as the worm is so small and filmy, hundreds or more might easily be overlooked. It is evidently a heterocotylean trematode, but does not seem to fit into any known genus. For the purpose of classification, it may be described as elongated, flat, two-mouth suckers, many testes, peculiarly armed cirrus, body towards posterior disc tranversely striated and spiny along dorsal surface with four claspers on opposite side, and with a fairly large terminal disc without suckers, but armed with two large hooks and several small ones. I would like therefore to suggest a new genus of the family *Gyrodactylidae* v. Ben et. Hesse.

The anterior end shows a mouth with two buccal suckers, much like those of a Microcotyle. Behind and between these is a small pharynx followed by a rather long gullet, but which is in no case

visibly divided into coeca, although a number of specimens were examined. The location of the genital pore is indicated by a flat plaque of amber colored chitin, and near it is seen a most peculiar head of the cirrus with a coronet of about twenty spines. (Fig. 49a.) This ornamental structure is at the end of a long tortuous vas deferens arising from a cluster of forty or more testes, which resemble those of the microcotylidae. The ovary is of a relatively fair size, situated a little anterior to the middle of the worm. It is, however, in the material at hand impossible to make out the whole female genital apparatus, although plenty of specimens, well stained, were under observation. The vitellaria were very plentiful and offered some obstruction to making out the anatomy. These glands extend from the genital pore on both sides throughout two-thirds of the worm's length. Along the dorsal surface for about one third of its length, the body of the worm showed its skin armed with sharp spines, looking chiefly towards the posterior and chiefly dorsal. On some portions also of the posterior part of the body, spines may be seen on the abdomen, and in this region of the spines, the body is seen to be crossed with transverse striae. An odd feature, too, is the presence on the abdomen just anterior to the disc of four relatively large claspers or chitinous suckers. These are oblong and of a different structure to those in the axine or microcotylidae. Still, they are of the same character. Three of these, the posterior ones, are placed near to and in advance of each other, while the anterior one is placed some distance anterior to the others, and it too seems to have at its base a flap of thin, semitransparent skin, which extends past the margin of the body.

The disc is a relatively large, saucer-like structure with two fairly large hooks attached to the under margin, and also along the edge of the disc are seen from the one side four other small hooks. If distributed evenly around the margin of the disc there would probably be in all eight of these small hooks. Only two large ones are seen, however. The egg seen in the uterus, is also odd. (Fig. 49b.) It is relatively very large with an anterior spike and a posterior filament. It is somewhat wrinkled.

Measurements of A. mirabile

Width	20μ
Disc diameter	33μ
Disc depth	15μ
Shield near genital pore	8μ long x 5μ wide
Egg length	15μ
Egg width	7μ
Length of spike	2μ
Filament at posterior end	15μ long
Across cirrus coronet	5μ

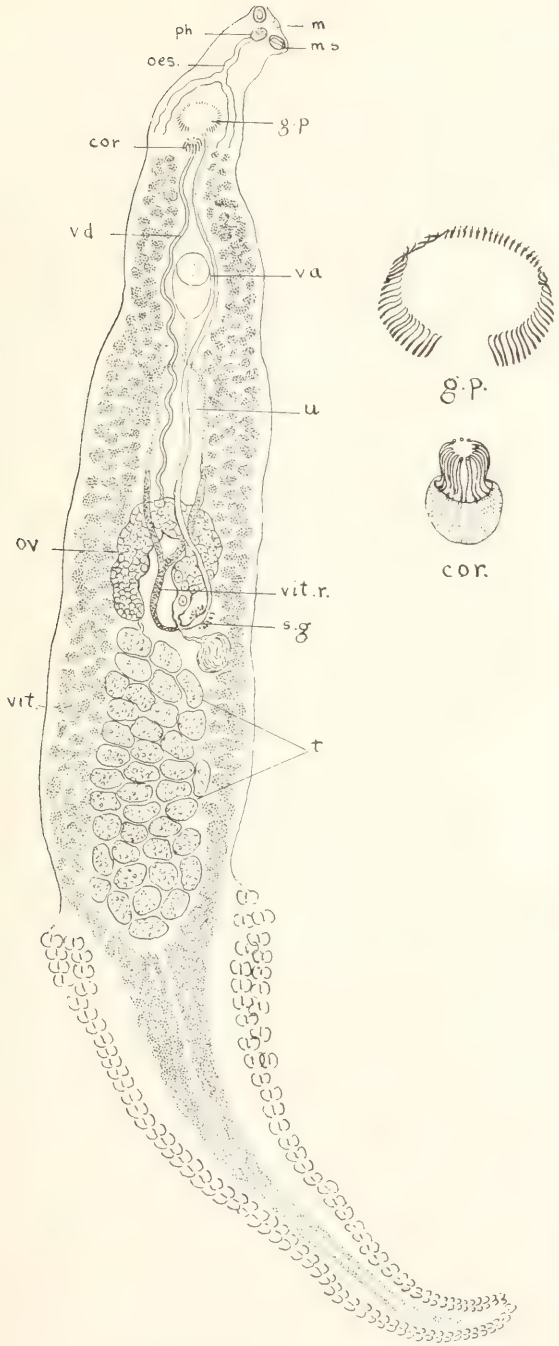

FIG. 50. *MICROCOTYLE SPINICIRRUS.*

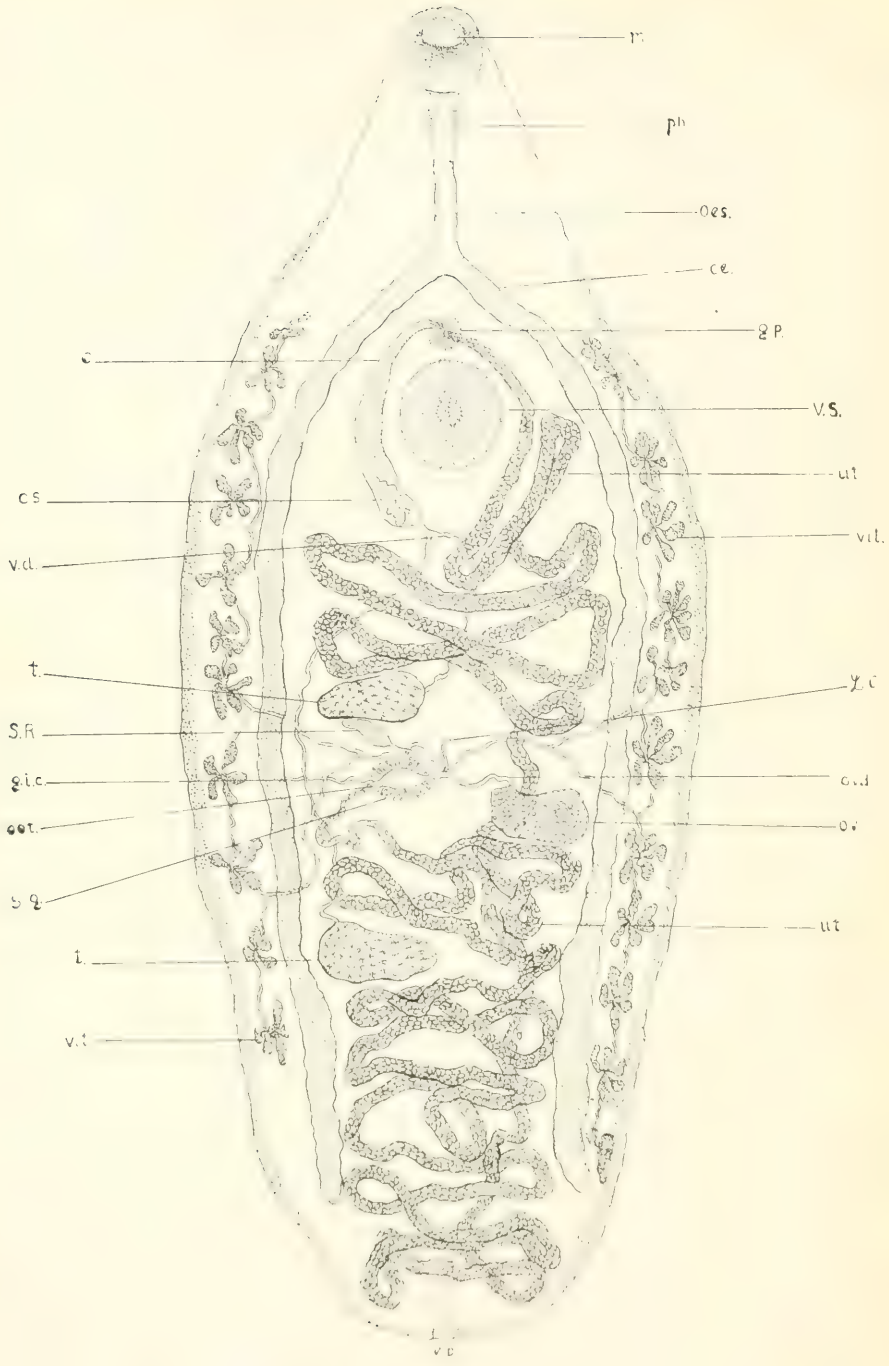

FIG. 8. — *LEUCOMIUM TROPIDONOTI.*

Microcotyle spinicirrus, sp. nov.

(Fig. 50)

Habitat—Gills of the fresh-water Drum. Found in lakes and rivers in North America.

On January 31, 1917, a fresh-water drum, *Aplodinotus grunniens* was sent me from the Aquarium at New York. It was a large fish and yet its gills were found so infested with microcotyle that they undoubtedly caused its death. The gills were of a cream color, although the fish had recently died. The worms had practically exhausted most of the blood.

This *Microcotyle* is not large and is rather gracefully formed. Its sucker disc is triangular in shape and as many as eighty or ninety suckers or claspers may be counted on it. The head is rather square at the anterior end with the mouth in the centre, and on each side of this is a buccal sucker. Between and posterior to these is the pharynx followed by a rather long oesophagus, which divides as usual into two coeca, and between these branches of the intestine the genital pore is seen surrounded by a large ring of hooks, counted in two or three instances there were fifty-four. (Fig. 50.) At one point toward the posterior they are absent for a short distance in the ring. The ring is 20μ across and the longest hooks are 5μ long. Immediately behind the open space in the ring is the anterior end of the cirrus, which is a peculiar structure as shown in Fig. 50. The vas deferens leading to it is very tortuous and wide. A short distance still further posterior, is a clear circular space, in the centre of which can be seen the vaginal opening, also surrounded by a cluster of hooks or spines which are small. The opening is dorsal and the vagina empties into the vitelline tubes which go to make the Y-shaped vitelline reservoir, from which a small tube joins the oviduct before the latter merges into the ootype as usual, though the duct from the seminal reservoir joins the oviduct first. The ovary itself is a horseshoe-shaped tube placed across the middle of the worm with the convex portion looking anteriorly. The testes are in the posterior end of the body, and are forty or fifty in number. The vitellaria are distributed along the sides of the body from near the genital opening almost to the tip of the sucker disc.

Measurements of Microcotyle spinicirrus

Length varies very much. An average would be 5 mm.
Width ..... 1 mm.
The disc about one-third the length of the worm.

Distomum tropidonoti, sp. nov.

(Fig. 51)

Host—*Tropidonotus trianguligerus.*
Habitat—Gall bladder.
Locality—Buitenzorg, Java.

On May 25, 1916, Dr. W. G. MacCallum found at Buitenzorg, Java, in the gall bladder of a *Tropidonotus trianguligerus,* a brownish-green snake, a solitary distome. Its mouth is sub-terminal, unarmed and is large, and it terminates in a short gullet or prepharyngeal oesophagus. The pharynx is roundish and is followed by the oesophagus, which is comparatively long. This, as usual, divides into the intestinal coeca, and in their angle is the genital pore, the cirrus and the uterus presenting here. In the centre of the body, nearly at the anterior quarter of its length is the acetabulum, the genital pore being in front of this. The ovary situated in the middle of the body, is not so large as either of the two testes, which are placed one behind it and one in front, and both on the opposite side, one in advance of the other. The vasa efferentia meet each other at right angles, at or near the base of the cirrus sac, one is short and the other very long. The vitellaria are peculiar, being placed along each side in open hand, shaped clusters and giving out numerous vitelline ducts, several of which concentrate at a small vitelline reservoir between the ovary and the anterior testis. The ducts sent toward the genital junction are very distinct, as are also the ducts connecting the lobulated masses. In this position may be plainly seen the genital junction. The seminal reservoir is placed beside the posterior surface of the ovary, and sends its duct to join the oviduct just in advance of the duct from the vitelline reservoir. The ootype is seen surrounded by the shell-gland. The oviduct is continuous with the uterus, which coiling near the posterior end of the body, passes anteriorly to the end at the genital pore. The seminal reservoir is very distinct and lies against the posterior side of the anterior testis. It is situated on the right side of the centre of the worm, but by means of a rather long duct, it empties into the oviduct. Laurer's canal is given off the oviduct very near the ootype, and proceeds a short distance anteriorly before passing through to the back. The genito-intestinal canal is given off from near the ootype, and proceeds to the right coecum, where it enters. The ootype is large and is surrounded by a profuse shell gland. The eggs are numerous, small, and yellow. The vagina can not be made out certainly. The skin is thick, unarmed, and somewhat corrugated, especially at the posterior end. The excretory pore is large and situated at the extreme posterior end of the body. The worm is only 5 mm. in length by 1.90 mm. in width.

Measurements of *Distomum tropidonoti*

Length	5 mm.
Width	2 mm.

Across mouth	20μ
Pharynx width	10μ
Pharynx length	10μ
Oesophagus length	30μ
Acetabulum	30μ
Ovary	30μ x 50μ
Testis	65μ x 30μ
Cirrus sac length	20μ
Egg	5μ x 2.50 μ

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Each author is responsible for the scientific accuracy and the proof reading of his contribution.

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Mr. A. Feinberg, artist, is also deserving of my sincere thanks for his patience and skill in preparing the plates.

FIGURES

ABBREVIATIONS

Abbreviations in explanation of plates:

a.—anus
b.c.—buccal sucker
b.—bursa
b.e.j.—bulbus ejaculatorius
c.s.—caudal sucker
c.s.—cirrus sac
c.p.—cirrus pouch
cl.—cloaca or genital pore
ce.—coeca.
cor.—coronet
c.—cirrus
c.gl.—cement glands
d.ej.—Ductus ejaculatorius
e.—eye
ex.p.—excretory pore
ex.v.—excretory vessels
g.i.c.—genito intestinal canal
g.p.—genital pore
h.—hook.
i.—intestine
li.—ligament
le.—lemnisci
l.c.—Laurers canal
m.—mouth
m.b.—muscular bands
m.g.—mucus glands
met.—metraterm
o.s.—oral sucker
ov.—ovary
oot.—ootype
oe.—oesophagus
ov.d.—oviduct
oes.g.—oesophageal gland
ph.—pharynx
p.p.—pars prostatica
pr.—prostate
p.—papillae post and preanal
res.—reservoir
s.g.—shell gland
s.v.—seminal vesicle or vesicula seminalis
s.r.—seminal reservoir
sp.—spicule
t.—testis
tr.—tridents
u.—uterus
u.g.—unicellular glands
v.d.—vas deferens
va.—vagina
vit.—vitellaria
vit.d.—vitelline duct
v.s.—ventral sucker or acetabulum
w.v.s.—water vascular system
y.d.—yolk duct
y.r.—yolk reservoir

STUDIES ON THE POLYSTOMIDA

Order—*Trematoda.*
Sub-order—*Heterocotylea.*
Family—*Polystomidae.*
Genus—*Polystoma.*

In 1808, when the order *Trematoda* was established by Rudolphi, it was made to include with other genera that of *Polystoma* (Zeder). In 1892, Monticelli proposed a new system, dividing the trematodes into three groups or sub-orders, the *Heterocotylea, Aspidocotylea,* and *Malacocotylea,* the first of which exactly coincides with the *Monogenea* of Van Beneden, and this arrangement has been generally adopted by recent authors (Pratt).

Thus, *Polystoma* (Zeder), according to this plan, will fall into the Sub-order *Heterocotylea,* Family *Polystomidae* Taschb. and Sub-family *Polystominae,* V. Beneden. These worms are usually found on the gills and in the urinary bladder of amphibians, and in the mouth, nose and urinary bladder of turtles. The posterior end has usually a disc, on which are situated six or more suckers. These suckers of polystomes consist of small, cup-shaped, circular discs, composed of muscular fibres in the wall, and arranged in various directions between the chitinous framework. These fibres are limited on the surface by a distinct membrane. The muscular fibres are so attached to the various parts of the chitinous framework that by their contraction, the cup-like cavity can be raised and narrowed so as to accomplish its sucking function. Other muscle fibres extend from the disc up into the body, so that the whole disc is under the control of the worm. The genus *Polystoma* is not always ectoparasitic since it is often found living in the urinary bladder of fish, amphibia or reptiles. It may also be sometimes found in the lungs and intestines. *Polystoma* have no metamorphosis. (Ray Lankester.)

The posterior adhesive appartus presents a variety in *Heterocotylea.* No doubt, the single sucker at the hinder end of the body represents the primitive arrangement. This sucker, which is always simple and never armed in the *Malacotylea* is usually multi-loculate in the *Aspidocotylea.* This posterior adhesive organ is usually formed of six, seldom of eight, suckers on a strong disc, and it is usually armed in the *Heterocotylea.* The anterior buccal suckers communicating with the oral cavity are usually present. The vagina, single or double, but someimes multiple as in the *P. integerrimum.* Common genital pore, median. The copulation of *Polystoma* has been observed (Zeller). It is probable that in the case of many of the *Heterocotylea* which live isolated on the gills of fishes, and where eggs are laid by them, they are able to self-fertilize themselves. This is borne out by observation

in *Distomum* species by Looss,, who finds spermatozoa within the uterus before the external pore is formed. They could, therefore, only have been derived from the ripe male organs, and further, in some species there is no penis, so that copulation could not have taken place. If self-fertilization may occur in a *Distomum*, there is every reason to expect its occurrence in *Heterocotylea* (Ray Lankester). "The eggs are laid in water, and when hatched the larvae swim freely in water by means of five incomplete girdles of cilia, of which the three anterior are incomplete dorsally and the two posterior, incomplete ventrally (Zeller)." "The young *Polystoma* makes its way from the mouth into the pharynx, and wanders along the alimentary tract to the rectum. On the formation of a cloacal bladder, the young *Polystoma* enters it. It is not till the third year that the parasite becomes sexually mature. The gill *Polystoma* parasites differ in several points from the cloacal form, notably in the absence of a vagina, and consequently in the impossibility of copulation." (Ray Lankester.)

"In *Polystoma*, each vagina opens through 20 or 30 small pores situated on the lateral swelling, and at its internal end, the vagina communicates with the transverse vitelline ducts, and in its course is sometimes dilated to form a spermatheca." (Ray Lankester). This latter statement does not seem to be quite correct, for many *Polystoma* are found with a single freely open vagina on each side, and without any lateral swelling whatever. "In the *Heterocotylea*, too, there is sure to be a narrow duct passing from the oviduct opposite the entrance of the vitelloduct to the right limb of the intestine. This genito-intestinal canal, whose true relations were discovered by Ijima, and since confirmed by Goto and all who have examined the matter, was originally called the internal vas deferens by von Siebold, and believed to be connected with the testis, close to which it passes. It was then looked upon as a means for direct internal fertilization. It appears to serve for the conveyance of superfluous yolk to the intestines which will serve as food." (Ray Lankester.)

I wish here to record in connection with these worms an observation made in the case of *Chrysemys scabra* and also in *Trionyx ferox*. During the examination of these turtles, I noticed that in each case there was a consolidation of the posterior fourth of one lung in the former, and one-third in the latter. In each case the diseased portion was removed and frozen sections made. The diseased tissue appeared to be a hardened mass of small round cysts, some containing cheesy matter, but the greater portion of them were cysts, which to the naked eye looked transparent. Even in the case where the egg with the brownish shall had been sectioned, I did not observe any segmenting or development. I was somewhat surprised to find that beside the inflammatory exudation and consolidation of the diseased lung, throughout the whole section were to be seen sections of brownish yellow, oval eggs, which resembled the eggs of *Polystoma*. In many cases merely the oval holes were left, where the sectioned eggs had dropped out.

I, at once, compared the measurements and found that *Polystoma* eggs measure in *P. digitatum* 30μ and 15μ, 25μ and 12μ and 25μ and 15μ, in *P. orbiculare* 0.21 and 0.24 mm. in diameter. In *P. aspidonectes* 20μ and 10μ, 15μ and 10μ. These were the two polystomes found infesting the turtles diseased. In the sections of diseased lung, the eggs measured 30μ and 20μ, 15μ and 10μ, none were larger and some smaller, as a rule those in the sections were smaller than the eggs measured in the mounted worms. However, the measurements are so close and the appearance of the eggs corresponds so closely with those in the worms, that one is forced to the conclusion that the condition of the lung in each case was owing to the deposit of great numbers of polystoma eggs. In one case a live *P. digitatum* was found in a bronchus very near the diseased mass. I also think that the sections showed in one or two instances the cross sections of the larval worm itelf. If this deduction is true, and I am sure it is, then the life history of *Polystoma* must be changed, at least in some instances. In each of the turtles examined, there were polystomes in the urinary bladder and also in the nasal cavities. In the one instance, that of *Trionyx ferox*, there were fifty or sixty worms in each location. This subject will bear further study, although I believe that what I have stated above will be found correct.

Polystoma troosti, sp. nov.

(Fig. 52)

Host—*Chrysemys troosti.*
Habitat—Urinary bladder.
Locality—New York Aquarium. Southern States.

On May 23, 1916, there were found in the urinary bladder of a Troost's terrapin, *Chrysemys troosti,* an odd *Polystoma,* which is unlike any other polystoma as far as I know, which has been hitherto described. There were only two specimens recovered, but as they are fairly perfect, they will serve for description. The general shape is as usual with these worms, an oblong, narrow at the mouth, wider at or about the middle of the body, and again narrowing before the clinging disc is reached. The mouth is distinctly terminal with thick lips, which are colored dark about the margins, in fact, this is the case throughout the whole body. The skin seems very dark from the presence of pigment. The pharynx, which is close up to the mouth, is bowl-shaped, and the external muscular fibres are directed around it, giving a rather ragged appearance. It is large and rather shallow, and is followed closely by the coeca or the intestines. These are wide and are soon lost in the vitellaria, which are very plentiful throughout the surface of the body, posteriorly quite to near the disc. A short distance posteriorly and between the coeca is seen the genital pore with a remarkably armed

cirrus and anterior to the opening. There seem to be three layers of hooks or spines, fourteen in the first layer, on the end of the cirrus, piled one on top of the other from a central point. The spines are long, and each is divided at its outer extremity or root into three or four filaments, all pointing posteriorly, or towards the body of the penis forming an unusually-shaped coronet. The vas deferens winds forward to form a seminal vesicle before reaching the genital pore. In the uterus near the genital pore, a relatively large round yellow egg occupies the distended organ. The surface of the egg is wrinkled from contraction. The ovary is small and elongated to about .40 mm. long by .10 mm. wide, and it is coiled upon itself on its way anteriorly. The egg is roundish and about .30 mm. in diameter. The testis is large, being .80 mm. in diameter. On the anterior surface of the ovary, a small seminal reservoir, or widening of the two vaginae at their junction, is seen, and from this point a tube leads to the oviduct. The shell gland can not be made out, nor is the uterus well defined, being distended out of all shape by the immense egg. The mouths of the vaginae may be seen among the vitellaria on each side opposite the genital pore. The vitellaria are unusually abundant throughout the body, rendering it difficult to trace the various organs. The vitelline ducts are plainly visible joining the oviduct. The clinging disc is rather irregularly shaped and supports six suckers. These are not quite like any others. They are surrounded on their margin by a flat, thick ring on which it is difficult to see the chitinous spines, they are so small. This whole ring seems to be lifted up from the structure below, making the sucker more deeply cupped. In the interior of the sucker, and surrounding its inner surface, there are a great number of small narrow finger-like processes, rods, or folds placed closely side by side and attached by their bases to the inner margin of the marginal flat ring, or else they may be attached to another ring just inside of the outer one. They are all directed towards the centre and are of about equal length, leaving the usual round hole in the centre or base of the sucker. They tend to keep the sucker in its concave shape and seem to terminate at another thickened ring which surrounds at a little distance the thin basal circular portion, and here is another marginal fringe of short free ends of folds which are somewhat like those in the central or concave portion of the sucker, only their inner or basal ends are free, and no doubt, their function is to close up the circular space and thus render the vacuum more perfect, for of course, it is in this way the suckers functionate under the influence of muscular fibres attached to the bottom of the sucker causing a vacuum. There is in the bottom of some of the suckers of this worm a small hook which is the only hook on the cotylophore. The flat marginal semi-elastic chitinous ring with its chitinous spines fixed on broad convex bases is without doubt, the foundation of these suckers in all polystomes. It keeps in shape the appliance ready at a moment's notice for attachment at the will of the worm.

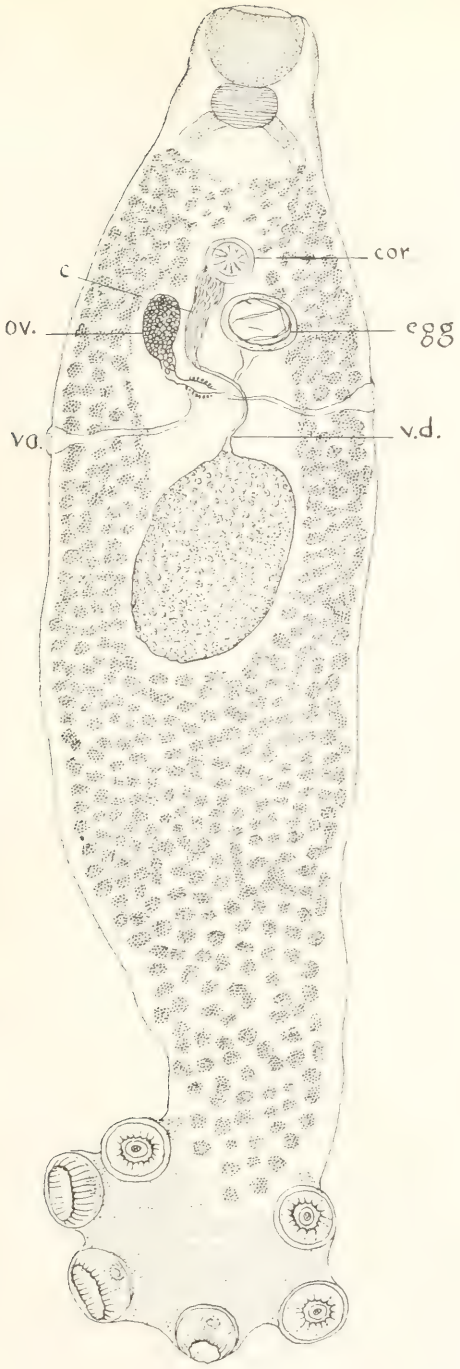

FIG. 52. *POLYSTOMA TROOSTI.*

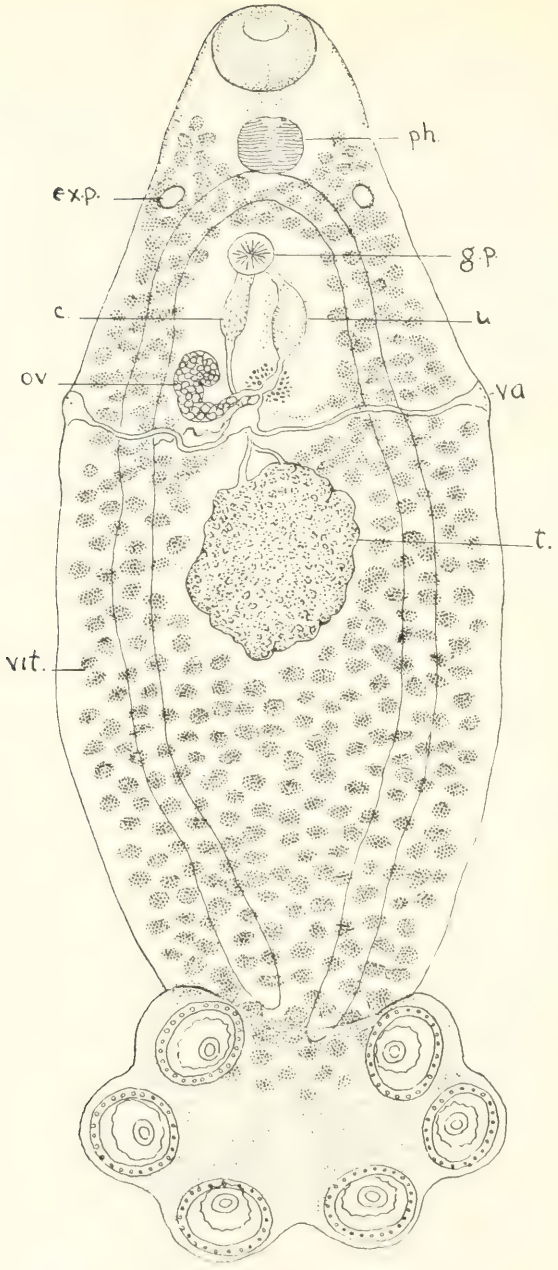

FIG. 58. *POLYSTOMA INTERMED.*

The water vascular system terminates in two pores one on each side a little anterior to the genital pore.

Measurements of P. troosti

Length	4.00 mm.
Width	1.00 mm.
Diameter of disc	.80 mm.
Diameter of each sucker	.20 mm.

Polystoma inerme, sp. nov.

(Fig. 53)

Host—*Chrysemys elegans.*
Habitat—Urinary bladder.
Locality—New York Aquarium.

From the host, Cumberland turtle, *Chrysemys elegans,* I am able to describe what appear to be three new forms of polystomes; viz.: *P. elegans, P. albicollis,* and *P. inerme.* At first I thought this must be a record, but I find that Stunkard had also been able to describe three forms from a host *Chrysemys marginata;* viz.: *P. orbiculare, P. megacotyle,* and *P. microcotyle.* The third and last worm found in the urinary bladder of *Chrysemys elegans,* is not large, but quite interesting. It is quite pointed at the head, and presents at the posterior end a disc which is as wide as the widest part of the body. It is wider from side to side than it is deep, and presents on its surface six suckers, which are placed three at each side. The first two being a little farther apart than the two posterior ones. No hooks whatever, are seen, and the disc itself is separated from the body by a distinct line which gives the impression that the disc is raised above the posterior end of the body, but this may be only muscular, for all of the specimens do not show this. The mouth is circular and subterminal, as usual, and is a short distance from the pharynx which seems to be connected by a short oesophagus. The pharynx is a circular muscular bowl closely connected with the intestine, which divides into two long coeca reaching quite to the disc. The cirrus and uterus present at the genital pore. The coronet in the three specimens before me is differently supplied with hooks, there being 16, 17, and 15. This would go to show that the number of hooks on the coronet cannot be taken as a point of diagnosis for they often vary in number. The cirrus sac is large relatively and the uterus is a good-sized fusiform sac, both presenting at the genital pore. The ovary is situated on the right side in all these specimens, and here I may say that it has been my experience to find these internal organs uniform, in the same positions in the body, as one would expect. The ovary is not large, it is club-shaped, the large end near

the side of the body cavity, and the small end the oviduct. This is short in its connection with the uterus. There are two vaginae very plainly seen, and although the eminences on the sides are not high, yet they are there and a number of entrances can be made out. Where these vaginae meet near the oviduct, they enter a small sac, from which a tube enters the oviduct. Here too, may be seen the canalis genito intestinalis emptying into the intestine. Laurer's canal can not be unmistakably made out as usual. The vas deferens arises in this case by two distinct branches, which shortly unite. The ootype is plainly visible, as is also the shell gland. The testis is a single, large, roughly rounded organ situated in the middle of the body. The vitellaria are very profuse, and extend from the pharynx to half way across the disc. In fact, these glands so cover the body, that only the two outlets of the excretory systems may be seen. These are large, one on each side anterior to the genital pore.

Measurements of P. inerme

Length	4.00-5.40 mm.
Width	1.50-2.00 mm.
Disc, wide	2.00 mm.
Disc, deep	1.20 mm.
Head	.60 mm.
Each sucker	.40 mm.
Testis	1.00 mm.

Polystoma albicollis, sp. nov.

(Fig. 54)

In the urinary bladder of a Cumberland turtle, *Chrysemys elegans,* there were found on January 20, 1917, three polystomes, which I describe below in the belief that they are new; if not, then it is the fault of my reference, and it does not matter.

They are as usual in the genus, elongated with a powerful adhering disc, fortified, in this case, with long hooks thereon; two long ones and two shorter ones between the two middle posterior suckers.

The mouth is somewhat sub-terminal, large and communicates with the pharynx by a short oesophagus. In front of the pharynx, the head is marked by a light colored band, forming a sort of neck. This is caused by the absence, in that particular zone, of the unicellular bodies and other cells. The pharynx is almost globular, the muscular fibres of which run in a more or less circular direction, giving it great strength. Just behind, on each side of this body is a large opening, the excretory pore. The intestinal coeca divide at the posterior lower border of the pharynx, and proceed backward under or among the profuse vitellaria,

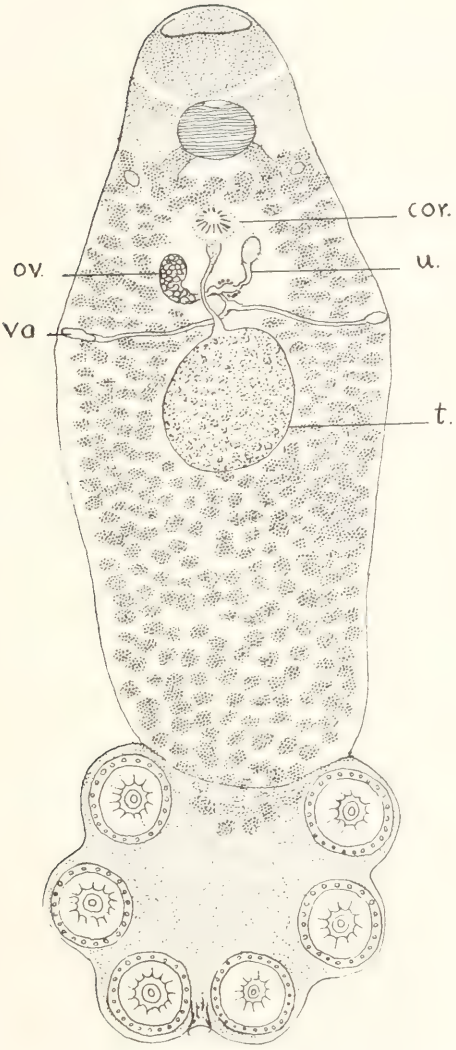

FIG. 54. *POLYSTOMA ALBICOLLIS.*

Polystoma elegans, sp. nov.

(Fig. 55)

Host—*Chrysemys elegans.*
Habitat—Urinary bladder.
Locality—New York Aquarium.

On January 20, 1917, there was found in the urinary bladder of a Cumberland turtle, *Chrysemys elegans,* a polystome which appears to be a new species. It is somewhat larger than those usually found in the urinary bladder of turtles, measuring nine mm. in length by two mm. in width at its widest part which is across the portion of the body where the genital junction is situated. The diameter of the clinging disc is two and one-half mm. Across the mouth it is 60μ. The mouth is sub-terminal and large, and presents apparently a small sucker on each side just within its margin. A short distance behind the mouth is the pharynx, which is fairly large. The intestinal ceca divide at once behind the pharynx, and in the angle formed is a mass of deeply-stained unicellular glands and a few masses of vitellaria.

Still further posteriorly is seen the coronet or armed end of the cirrus which is large and very prominent. The armature consists of a circle of hooks or spines, seventeen in number, which, at their outer extremity, are divided each into two or three small branches, in fact, in some there are six or eight branches, making each spine look like a miniature brush. These are the roots for muscular attachment. Each spine is 20μ long and encloses a circular space. The whole end of the cirrus measures 75μ in diameter. A very short distance posterior to this is the vesicula seminalis, which is pear-shaped or fusiform. The small end being directed posteriorly in the form of the vas deferens which is seen coming from the anterior end of the testis. Behind and to the left of the cirrus head is a relatively immense egg, which is yellow, roundish, and somewhat wrinkled; longest diameter, 120μ. It seems to occupy the whole uterus, which in these worms is only capable of holding one egg at a time. Behind the uterus and to the left, is seen the ovary which is oblong, .60 mm. x .20 mm. and three times as long as it is wide. It is placed somewhat obliquely across the body and to its left, on the side of the worm, is seen the single prominence which indicates the presence of the many openings into the single vagina. The other vagina may be present but cannot be made out. The testis is an oval large body, 1.60 mm. long by .80 mm. wide. The vitellaria are exceedingly abundant, and present a general tube, almost surrounding the central portion of the body. Some of the vitelline masses extend into the sucker disc. The genital junction is situated between the ovary and the uterus, but no seminal reservoir or shell gland can be seen.

The sucker disc, 2.20 mm. in diameter, has on its surface six suckers which are different from those of other polystomes. They are cir-

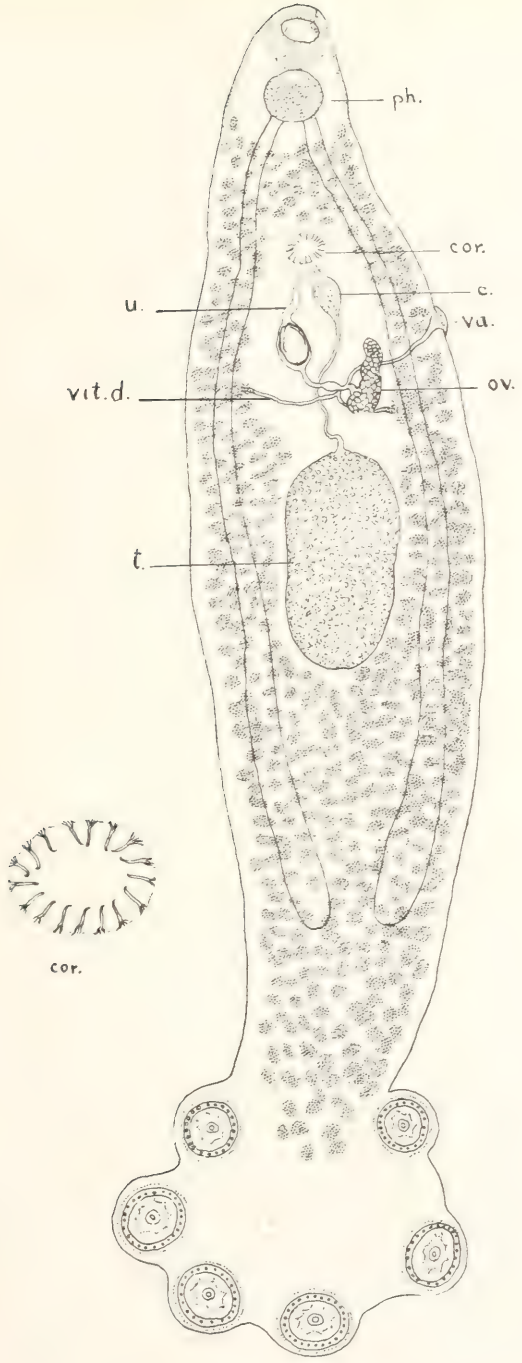

FIG. 55. *POLYSTOMA ELEGANS.*

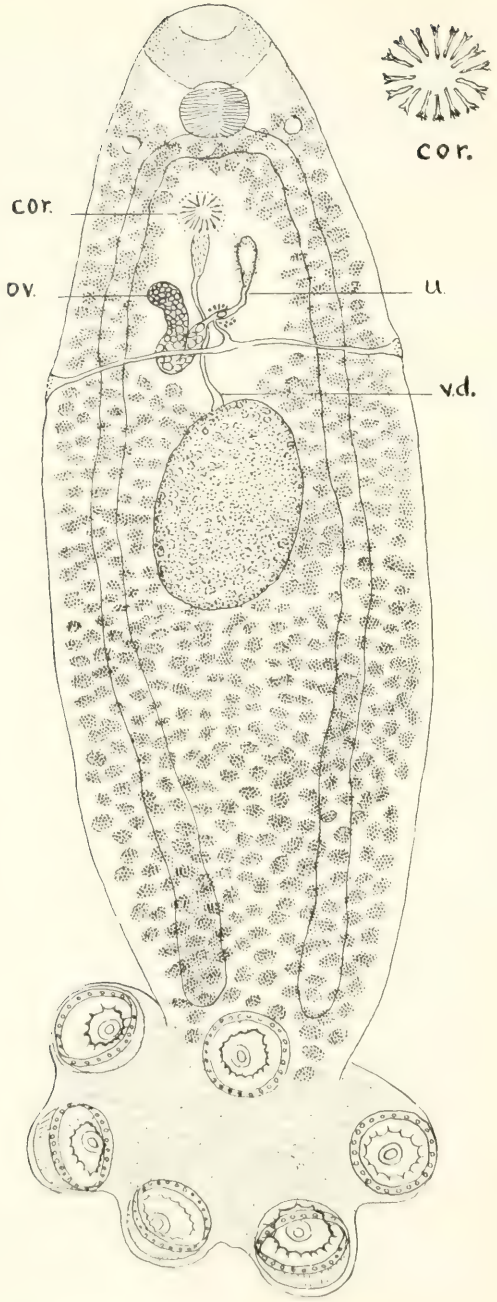

FIG. 9a. *POLYSTOMA SPINULOSUM.*

cular and deeply cupped, and are surrounded by the usual flat chitinous ring, on which, at equal distances from each other, are forty-eight little spines attached to the ring by a circular base, which is convex and much wider than the spine. In the centre of the bottom of the cup is a circular opening only covered by a membrane, and surrounding it is another thickened ring. Extending from the inner margin of the large outer chitinous ring is a membrane which extends toward the bottom, having its central margin irregularly torn as it were, not appearing in the forms of regular processes, as in some polystomes. The sucker disc is an irregular-shaped block, and the six suckers are placed well back from its margin. The suckers measure .40 mm. in diameter. There are no hooks whatever on the disc nor is there one in the bottom of the sucker.

Polystoma spinulosum, sp. nov.

(Fig. 56)

Host—*Chrysemys picta.*
Habitat—Urinary bladder.
Locality—New York Aquarium.

The above trematode was found in the urinary bladder of a painted turtle, *Chrysemys picta,* on December 18, 1912. As far as could be observed it was solitary. It has some peculiarities which others of this genus have not, so that it is thought proper to put it on record as a new species. The mouth is large and situated at the bottom of a terminal cup-shaped depression, the lips of which are rather thick. The oesophagus is not long and terminates in the usual bowl-shaped pharynx, which, in almost all of these worms, is the powerful suctorial apparatus. In front of the pharynx and surrounding the head of the worm is a ring, which is whiter than the rest of the region, giving it the appearance of having a sort of collar, which is conspicuous. The intestines start from the lower and back part of the pharynx and extend almost to the disc posteriorly. In the angle of the division of the coeca, the conspicuous head of the cirrus is seen, armed as usual with irregularly-shaped spines, or hooks, which are somewhat clubbed and split at their outer extremities or roots for muscular attachment. Behind this and almost in contact with it, is the pear-shaped uterus with the base toward the genital pore which is at the cirrus head. Toward the other side of the body, or the left side, is the ovary which is elongated and curved, and behind this is the single testis which is very large, perhaps four times as large as the ovary. From the anterior end of the testis is seen the vas deferens, which coils dorsal to the ovary and ends in a cirrus sac, which encloses the cirrus. From the left, lower side of the ovary, the oviduct arises and coils upward and forward to terminate in the uterus. In this case, the genital junction occurs dorsal to the pos-

terior end of the ovary, and the ootype and shell gland are plainly visible. There are two vaginae, which are situated one on each side, opposite the lower or posterior end of the ovary. They are both only large uneven openings and the vaginae meet from each side to connect with the oviduct. The vitelline ducts can not be made out, but they must also terminate near the same location. The vitellaria are very profuse and cover the most of the body from the pharynx to the disc.

The disc is an unusual structure. There are six suckers, each having on the surrounding, flat, chitinous ring about forty chitinous spines attached to a circular placque, while between each pair, there are one or two hair-like bristles or spines, which would tend to give greater adhesive properties to the sucker. There is the usual small sucker disc at the bottom of each of the main suckers. There are many muscular bands which run from the disc up into the body of the worm, by which it may be controlled.

Measurements of P. spinulosum

Length	5.60 mm.
Width	1.80 mm.
Disc	2.00 mm.
Testis	1.00 mm.
Head	.40 mm.
Ovary	.40 mm.

Polystoma chelodinae, sp. nov.

(Fig. 57)

Host—*Chelodina longicollis.*
Habitat—Urinary bladder.
Locality—New York Zoological Park (from Australia).

This small trematode was found in the urinary bladder of a small long-necked turtle, *Chelodina longicollis*, Australia, and obtained from the New York Zoological Park.

There is nothing unusual about its conformation. The mouth is subterminal and the lip is unusally thick. The orifice in life was surrounded by numerous papillae, probably folds of the lip, which have disappeared under pressure. The pharynx is very large relatively, and the coeca divide at its back. These latter are large and extend almost to the posterior disc. The genital pore is situated some distance posterior to the division of the coeca, and is overhung by the cirrus head, which has a coronet of 15 hooks. The uterus is an oblong bag, presenting its mouth at the genital pore. The ovary is situated at the junction of the anterior and middle thirds of the body on the right side, and its oviduct first runs posteriorly until it meets the duct from

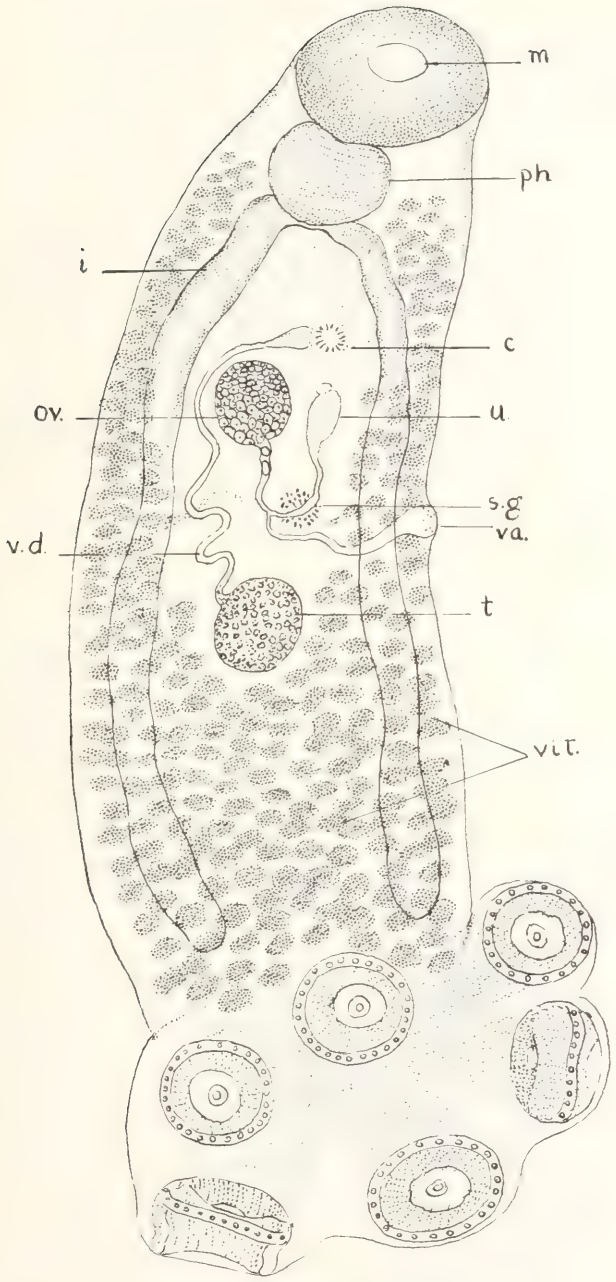

FIG. 57. *POLYSTOMA CHELODINAE.*

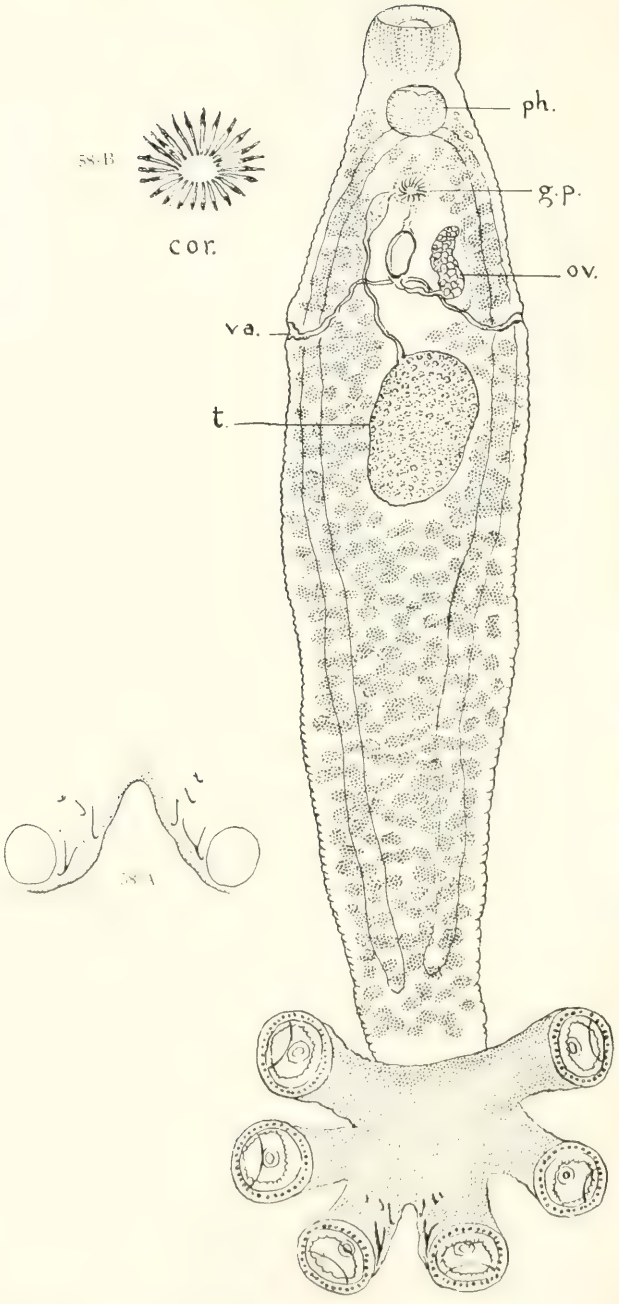

FIG. 58. *POLYSTOMA DIGITATUM*. FIG. 58-A. DETAIL OF HOOKS ON DISC.
FIG. 58-B. CORONET OF CIRRUS.

the vagina, which opens about the middle of the left side on an eminence. No seminal reservoir is seen.

The testis is large and round. The vitellaria very plentiful. The clinging disc is short, but wide and extends beyond both sides of the body. It is not more than two-thirds as deep as it is wide, and the suckers are placed in two parallel lines across the diameter of the body. The suckers are large and apparently of the usual construction. There are no hooks whatever on its surface.

Measurements of P. chelodinae

Length	3.50 mm.
Width	1.00 mm.
Disc	1.75 mm.
Head across	60μ
Testis	40μ
Ovary	20μ
Mouth across	30μ
Pharynx	35μ
Suckers	30μ
Egg around	10μ
Coronet	15 hooks

Polystoma digitatum, sp. nov.

(Fig. 58, 58a and 58b.)

Host—*Aspidonectes spinifer.*
Habitat—Nasal cavities.
Locality—New York Aquarium.

In the nasal cavities of a soft-shelled turtle, *Aspidonectes spinifer* (Le Sueur), there were found on February 5, 1913, three trematodes which, as far as I know, are of a new species.

The worm is not large, quite pointed at the head, but the most conspicuous feature is the relatively large posterior clinging sucker disc, which, as is very common with the *Polystomidae* bears six suckers. These latter are also peculiar, inasmuch as they differ from other species in being situated each at the end of a digit, while in others the sucker is placed directly on the surface of the disc block, each being deeply cupped.* In this species, the disc is something like that of the *Diclidophora.* The suckers themselves are quite different, for each is surrounded by the usual flat chitinous ring in which are placed a great

*This species has since also been found in a Southern soft-shelled turtle, *Trionyx ferox;* as many as fifty were found in the nostrils of one turtle. The specimens were larger.

number of chitinous spines equidistant from each other, and with a small hook in the base of each. Muscular strands, which control the suckers, can be seen extending up into the body. The enclosed cup-like cavity has a circular-covered opening at the bottom, but attached to the inner margin of the flat ring is a membrane which almost encloses the cavity, only a semi-circular opening being left at one side. The disc, itself, is marked off from the body by a ridge indicating the attachment, the body extending dorsally to the disc. The anterior end or mouth is cup-shaped with thick lips capable of extension. The mouth proper appearing at the bottom of the depressed portion. A gullet or oesophagus may be seen extending to the pharynx which is a bowl-shaped muscular structure, situated just posterior to what is a neck marked out by a narrow band or ring, which is almost devoid of the usual cells in the parenchyma which stain. The intestinal coeca are not very evident, being enclosed by the vitellaria and highly-colored stained tissues of the body. In the center of the body, within the angle of the ceca and a short distance posterior to the pharynx, is seen the prominent head of the cirrus surrounded by its armature of 31-35 spines. There are sometimes thirty-six of these spines, and they are of peculiar shape, being long and very slender at their base, but toward the outer extremity or root being much thickened by the divisions, which lie close to the center or stem and give attachment to muscular fibres. They, too, are directed outward and backward. The vas deferens which rises from the anterior end or side of the roundish single testis, winds up along the right side of the worm, just inside of the vitellaria to terminate in a cirrus sac, in which the cirrus is enclosed up to the edge of the genital opening. The uterus, a more or less pear-shaped, hollow structure with the mouth at the base directed forward toward the genital pore, is not large and receives the coiling oviduct from the ovary. This is not long, but is seen to receive the vagina and also the vitelline ducts. No shell gland can be made out, nor is there a seminal reservoir to be seen in the course of either of the vaginae. The genital junction is plainly seen between the anterior end of the testis and the ovary a short distance in front of it. The testis is round and about three times as large as the ovary. The vaginae open on each side of the body, a little in advance of the level of the anterior edge of the testis. The vaginal orifice is a large corrugated single opening without any prominence on the surface of the skin. The vaginae can be seen making their way to join the oviduct. This is perhaps unusual, but is certainly present.

For classification, worm is elongated, skin thick and corrugated. The vaginae not arising on eminence on each side, large clinging disc with each sucker placed on a digit.

Measurements of Polystoma digitatum

Length	4.00 mm.
Width	.80 mm.

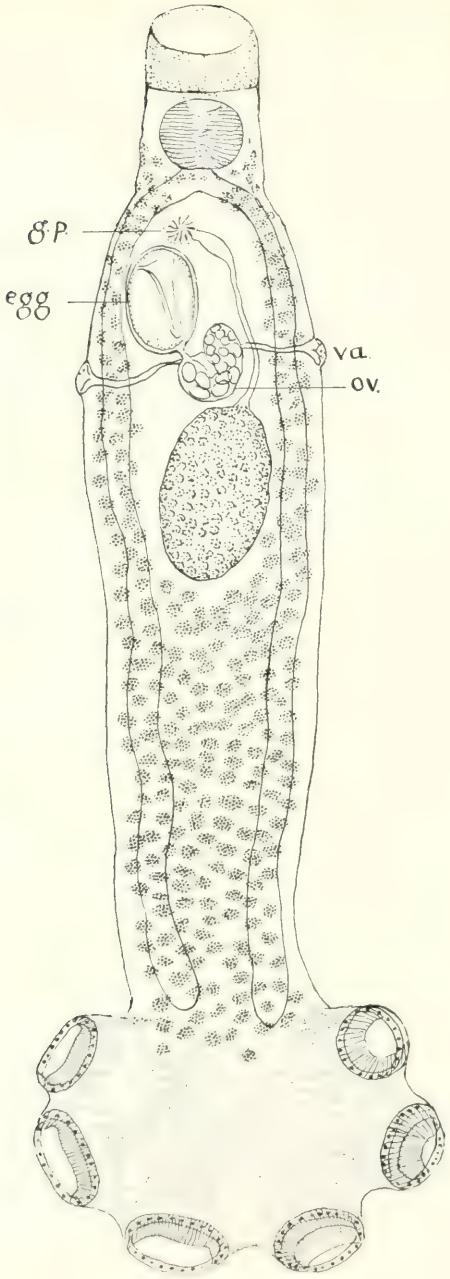

FIG. 89 *POLYSTOMA ASPIDONECTIS.*

Disc	1.40 mm.
Testis	.60 mm.
Ovary	.30 mm.
Pharynx	.20 mm.
Length of digit (including sucker)	.40 mm.
Diameter of sucker	.30 mm.

Polystoma aspidonectis, sp. nov.

(Fig. 59)

Host—*Trionyx ferox.*
Habitat—Nasal cavities, lung and intestines.
Locality—New York Aquarium.

On May 10, 1916, these trematodes, of which there were collected five from the same host; four were from the intestine and one from the lung. From the same host, soft-shelled turtle, *Trionyx ferox*, there were also collected from the nasal cavities four larger forms which I have ventured to name *P. rugosa* for the reason given in its description. It seems rather odd that while these worms are usually found in the nasal cavities or urinary bladder, they were in the case of *P. aspidonectis* found in the intestine and lung and not in the nostrils, although they might easily have wandered away from the natural habitat, if they were not driven away by *P. rugosa.*

P. aspidonectis, as will be seen by the measurements, is small, probably one of the smallest polystomes yet observed. To prove that they are adults, one has only to notice the relatively immense egg which distends the uterus, apparently to its fullest capacity. The mouth is rather large and more or less terminal, and round in shape. The pharynx is but a short distance posterior to the mouth, and is bowl shaped, almost round, and the intestinal ceca seem to be given off at once. Their course, however, can not be seen or followed on account of the vitellaria and dense cellular structure in this region. In the centre of the body, and a short distance posterior is seen the very prominent armed head of the cirrus. It has seventeen rather long spines, which concentrate from outward and backward. Each spine is branched at its outer extremity, and the whole number are piled on one another and are somewhat tangled together. The vas deferens, which leads from the anterior surface of the large oval single testis, passes dorsal to the uterus containing the egg. The uterus which is situated immediately behind the cirrus head, where presumably the genital pore is, cannot well be made out, for it can only contain one egg at a time and is distended to such a thin sac that it can not be seen, although the oviduct may be seen leading up to it from the ovary.

The ovary is rather small, situated in front of and to the left of the testis and about one-quarter or one-third of its size. On each side of the worm and almost on a level with the anterior edge of the ovary, may be seen the raised openings of the vaginae. These lead backward and inward to join the oviduct, but no dilatation indicating a seminal reservoir can be seen. The vitelaria are plentiful along the sides of the worm, and the vitelline ducts can be seen proceeding to join the oviduct between the testis and the ovary, where is situated the genital junction. The disc is the most prominent feature of the body, situated at the posterior end, it is a more or less quadrangular block, on which are placed six suckers of peculiar formation. They are cup-shaped as usual, surrounded on their margins by a flat chitinous ring, upon which may be seen thirty-two spines, placed at equal distances from each other around the whole circumference, and between each pair of spines, there appears in some cases quite plainly a Y-shaped hook. This may not be certain, on account of the material at hand, but the appearance is strongly suggested. Inside the sucker at its bottom, is a circular-covered hole as usual, which is surrounded by a membrane in folds, the outer margin of which is attached to the marginal chitinous ring. No large hooks are seen on the disc as in the *P. integerrimum,* but there is a small one at the base of each sucker.

For the purpose of classification, the body may be said to be long, narrow and straight; cirrus with an armature of seventeen hooks, two vaginae, egg almost oval, large, and yellow.

Measurements of P. aspidonectis

Length	2.60 mm.
Width	.40 mm.
Disc	.80 mm.
Egg	.20 mm.
Testis	.60 mm.
Ovary	.20 mm.

Polystoma rugosa, sp. nov.

(Fig. 60)

Host—*Trionyx ferox.*
Habitat—Nostrils.
Locality—New York Aquarium.

Obtained from the nostrils of a Southern soft-shelled turtle, *Trionyx ferox,* on May 10, 1916. This *Polystoma* is small, short, and rather broad, anterior end wide in proportion to length, however, the four specimens under observation have all a peculiar corrugated skin. In fact, the skin appears thick and is thrown into more or less transverse folds which extend from the neck to the sucker disc, giving the

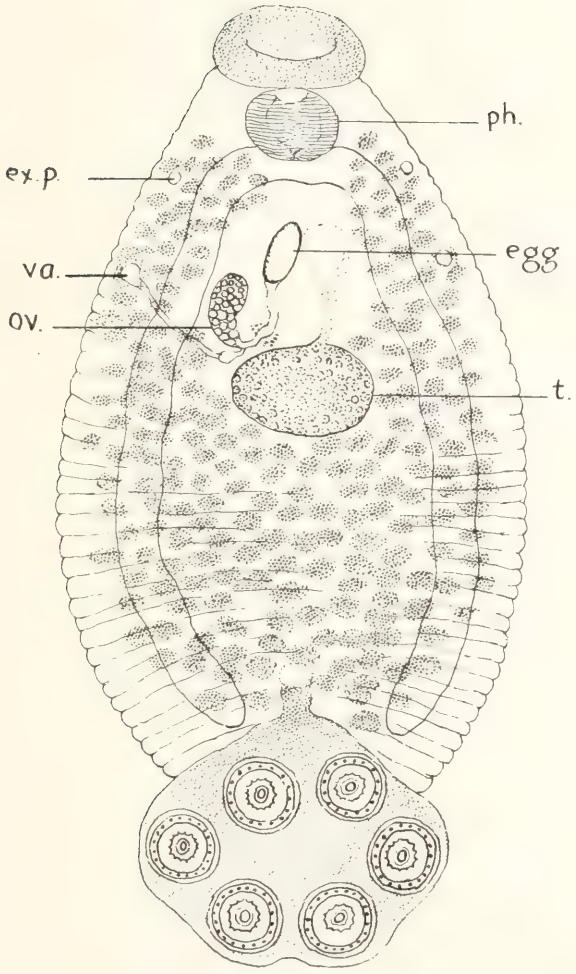

FIG. 69. *POLYSTOMA RUGOSA*.

impression that the whole body may be capable of great extension. They were found in the nostrils of their host.

The mouth is large and, terminal with the bowl-shaped circular pharynx close to it, in fact, immediately posterior to the mouth and apparently, but a continuation of the same. The dorsal edge of the mouth or lip is very prominent. There is but the shortest possible neck. The pharynx is almost as wide as the mouth and posteriorly it empties immediately into the intestine, there being no oesophagus. The intestine branches almost immediately behind the pharynx, which indeed may be said to enter the intestine. This latter structure is very wide and extends in a wide arch to each side, and proceeds posteriorly to the anterior margin of the sucker disc on each side, but does not seem to be continuous, although the cecal ends come closely together. Between these ends, and a little anterior to them may be seen a large opening, the excretory pore, the posterior edge of which seems to be connected with the disc by a rather wide band. This band-like structure may be a wide tube, which plainly appears to connect with the base of each of the first suckers. The edges of this tube are also corrugated. The disc is a more or less quadrangular body with six cup-shaped suckers, so placed that there are two side-by-side next to the body, and four others, situated around the edges of the distal margin. They are of an equal size and are circular. Around the margin of each, which is in the form of a flat chitinous ring, there are about thirty-two small spines, each set on a small chitinous placque on the margin of the ring. Inside of this is another ring, to which is attached, what apears to be a membrane with fibrous striae radiating rfom its outer margin, forming an opening in the center, and in the center of this and of the sucker is a round-covered opening which is again surrounded with a thickened margin. The whole disc is seen to be surrounded by a narrow membranous edge. Within the arch of the intestines is the genital aperture, where the mouth of the uterus is seen, and in one case there is present a large, yellow, fusiform egg without any polar filaments. Behind the genital pore is the large, single, more or less oval testis, placed directly across the body. The ovary is small and oblong, and is situated in front of and to the right of the testis, lengthwise of the body. The uterus is a more or less conical sac, capable of holding one egg at a time. Cirrus has no coronet, at least it is not seen in any of the specimens.

On each side, a little posterior to the level of the genital pore may be seen the vaginal orifice, a rather large, round, open pore, and in front of these on each side an excretory pore. The vitellaria are quite plentiful, extending in golden-colored masses throughout most of the surface of the body. No hooks are seen between the two central posterior suckers or elsewhere in many specimens of this worm examined.

On further study of these worms, I would say that until more material can be obtained so that the body may be sectioned, there are some

points which ca not be cleared up. For instance, no cirrus or coronet can be seen in any of the four worms, one worm being spoiled and another so thickly studded with escaped vitelline material that the internal organs cannot be studied. In the remaining two worms, which are perfect specimens, the uterus is occupied with a very large oblong egg obscuring the genital pore in one case, and the very large testis in each almost covers the entire field in this important region. Then it must be said that the corrugated skin extends across the body, in coarse ridges and at the junction of the disc with the body, a deep groove seems to almost separate it from the body, although the mentioned wide band or duct from what appears to be a large excretory pore is not affected, but plainly retains its position.

Measurements of P. rugosa

Length	4.00 mm.
Width	2.00 mm.
Width of disc	1.40 mm.
Width of mouth end	.40 mm.
Egg length	.40 mm.
Egg width	.20 mm.
Testis length	.60 mm.
Testis width	.30 mm.
Ovary length	.20 mm.
Ovary width	.15 mm.
Diameter of suckers on disc	40μ
Width of mouth	30μ
Width of pharynx	45μ

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ZOOPATHOLOGICA

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NOTES ON THE GENUS CAMALLANUS AND OTHER NEMATODES FROM VARIOUS HOSTS

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Mr. A. Feinberg, artist, is also deserving of my sincere thanks for his patience and skill in preparing the plates.

FIGURES

ABBREVIATIONS

Abbreviations in explanation of plates:

a.—anus
b.c.—buccal sucker
b.—bursa
b.e.j.—bulbus ejaculatorius
c.s.—caudal sucker
c.s.—cirrus sac
c.p.—cirrus pouch
cl.—cloaca or genital pore
ce.—coeca.
cor.—coronet
c.—cirrus
c.gl.—cement glands
d.ej.—Ductus ejaculatorius
e.—eye
ex.p.—excretory pore
ex.v.—excretory vessels
g.i.c.—genito intestinal canal
g.p.—genital pore
h.—hook.
i.—intestine
li.—ligament
le.—lemnisci
L.c.—Laurers canal
m.—mouth
m.b.—muscular bands
m.g.—mucus glands
met.—metraterm
o.s.—oral sucker
ov.—ovary
oot.—ootype
oe.—oesophagus
ov.d.—oviduct
oes.g.—oesophageal gland
ph.—pharynx
p.p.—pars prostatica
pr.—prostate
p.—papillae post and preanal
res.—reservoir
s.g.—shell gland
s.v.—seminal vesicle or vesicula seminalis
s.r.—seminal reservoir
sp.—spicule
t.—testis
tr.—tridents
u.—uterus
u.g.—unicellular glands
v.d.—vas deferens
va.—vagina
vit.—vitellaria
vit.d.—vitelline duct
v.s.—ventral sucker or acetabulum
w.v.s.—water vascular system
y.d.—yolk duct
y.r.—yolk reservoir

NOTES ON THE GENUS CAMALLANUS AND OTHER NEMATODES FROM VARIOUS HOSTS

Parasitic nematodes are by far among the most important parasites affecting human welfare and disease. When we consider their prevalence in domestic animals, and fish which constitute the most important article of human food, not to speak of their parasitism in vegetable food stuffs, we are easily led to see their importance in the economy and welfare of mankind. It is consequently remarkable how little they and their influence have been studied, even from an economic point of view. The field is so large and so comparatively unworked, that it is difficult to decide where to continue the study. Within a short time the writer has met with a form of nematode, which has been but little noticed on this continent, in fact the only reference known is one made by Prof. H. B. Ward in a contribution from the Zool. Lab. of the University of Ill., No. 78, 1916, in which he describes very generally the occurrence of two new species of *Camallanus* under the names *C. oxycephalus* and *C. ancylodirus*. Railliet and Henry in 1915 took up the study of these parasites in Europe and established for them a new genus. Hitherto, while they had been observed, no definite effort other than as given above had been made to give them a standing in the biological world. Dr. Ward claims that his two species, one found in a German Carp and the other two in a white bass and a black Crappie, all hosts from Iowa, are the first described in America, and from the general tenor of his paper one would infer that the worm was rare. I am glad to give my experience with this parasite, which I think will indicate that it is anything but rare, and this I do in as few short notes as possible. I may premise by saying that for the last seven or eight years the Director of the New York Aquarium has kindly placed a large amount of material at my disposal, which was collected from distant points in America. Other sources also have been made available.

The genus *Camallanus* is a haematophagic nematode whose habits appear to be those of the *Strongylidae*, in fact it would appear that it should belong to a sub-family of that genus, instead of being established as a separate genus. They live in the same organs, have identical habits and attach themselves to the mucous membranes in the same manner as is done by the *Strongylidae*. They have a muscular pharynx or upper part of the oesophagus which enables them to suck the blood from the mucous membrane after it has been wounded by the peculiar

structure of their mouths, which is a hollow surface with long sharp ribs or knives, which can be brought together much as the hookworm does with its sucking appartus, not excepting the buccal sacs which may be slightly different in shape, but identical in function. Beside this, they are probably more fertile than hookworms, since the whole body of the female is a distended sac of living larvae and eggs, and the females are about fifteen or twenty times as plentiful as the males.

The adult worm is red and varies in length from 15 mm. to 25 mm., and in width about .25 to .30μ. The body is very delicately formed, gradually tapering to the posterior end where the tip is generally trifid. The female is much larger than the male, and as a rule the shoulders of the female are much more angular or square than in the case of the male. However this may not hold in all cases, but it seems to be the rule. The vulva is situated on the ventral surface very near the middle of the worm. It is usually very prominent and swollen into two lips as shown in Fig. . The head, however, is the most prominent feature, and unlike that of the hookworm, is a very ornamental structure. It is chiefly composed of chitin and is shell-like in shape, being quite concave with six main ribs on each side of the central line, which converge toward the throat. There are also a few short ribs which help to give this part of the mouth at its back a hollow fan-shaped appearance. On each side of this formation is a very decided concave portion, which answers to the buccal capsule of the hookworm. In action the mouth seems capable of closing from each side, while the sharp-pointed rods make a wound in the mucous membrane, or one side crosses behind the other as in a pair of hair clippers. The powerful muscular pharynx or oesophagus, exactly like that of the strongylidae, then performs its sucking action. The whole head of the worm is often found buried in the mucous membrane, but it may be easily detached. The whole head, including when present the two or three lateral pendants, called by Ward "tridents" is composed of amber-colored chitin and across the throat is a straight band of the same material. The neck is straight There is also on each side on the dorsal surface of the ribs or knives a bar of chitin across the anterior ends of the median four of each half, for the purpose, possibly of stiffening them. Usually, there are five or six long ribs which reach from the anterior lip and converge in the throat, and between them at their anterior ends are two short ribs on each side of the median line.. The two central ones are short and very like sharp teeth. The extreme margin of the head is bounded by transparent material with three considerable nodules, one on each side and a small one in the middle. The anterior end of the oesophagus extends quite to the throat. The length of the muscular portion of the oesophagus is usually about 55μ, width at the widest part 20μ. It is bottle shaped. The second part of the oesophagus is 90μ long and generally the outside is ragged. The protruded flaps of the vulva of the gravid worm measure 35μ x 15μ. The mouth of the vagina is 5μ wide. Across the head 20μ, across the shoulders

40μ, across the mouth, which is always patent 15μ, and length of the "tridents" 10μ. Across the gravid uterus 40μ. Anus is within 40μ of the tip usually. No doubt these worms vary in size in different hosts, but the above measurements are those of a full-sized gravid female. The uterus usually contains both larvae and eggs, but the former are in thousands. They measure 20μ long x 2μ wide. The male is a small worm beside the female, where the females are 20 or 25 mm. long the males average 10 to 15 mm., and about half the width of the female. The posterior end of the male is usually coiled, and two white cirri may be seen protruding or within the body. They are about 50μ long and equal in length. There are three pairs of post anal papillae, and one pair of preanal. These are not easily seen for the tail is so closely coiled. Near the anus, probably on each side of it, is a semicircular flap, which acts like a bursa in the bursated nematodes. In all these worms there are to be seen the head glands as described by Looss in his paper in 1901. "The Sclerostomidae of Horses and Donkeys in Egypt" (Rec. Govt. Med. School, Cairo). The skin is finely striated.

Camallanus oxycephalus, Ward.

Locality—New York Aquarium.

On November 4, 1917, in the stomach of a Mississippi alligator, there was found an adult female specimen of this species *Camallanus oxycephalus* (Ward).* The general description given will answer in this case, except that near the anus there is a constriction in the bowel, opposite which on the outside of the gut on each side there is a tag like process both pointing toward the tail.

Camallanus scabrae, sp. nov.

(Fig. 61)

On December 14, 1917, there were found in the intestine of a *Chrysemys scabra* and six collected, two males and four females, of these worms. More could have been recovered if necessary. They possess no peculiarity beyond the general description, except that perhaps they were somewhat smaller.

Locality—New York Zoological Park.

On December 21, 1917, there were found in the intestines and stomach of a *Chrysemys scabra* a great number, in fact there were hundreds of the worms, many of them still clinging fast to the mucous membranes. Proportion of males about two to ten females. Females about 20 mm. long. Males about 15 mm. long. They have a peculiar

*"The Journal of Parasitology," Vol. III, No. 2, Dec., 1916.

bursa. Two post anal papillae and six preanal papillae. They have two prominent spicules.

Locality—New York Zoological Park.

also

On June 25, 1916, there was found in a *Chrysemys picta* an adult, gravid female *Camallanus scabra*. This one also shows the anal valve. Head glands, very distinct. No peculiarity. Size about 20 mm. long.

Locality—Woods Hole, Mass.

Camallanus troosti, nov. sp.

On May 25, 1916, there were found in the intestines of a Troost's terrapin, *Chrysemys* troosti, a lot of large *Camallanus* worms, six females and one male were collected and mounted. The male presented about 20μ in advance of the anus a bottle-shaped cyst 10μ x 8μ, on the outside, and from it a tube extended into the body, evidently a part of the bursal sac. The cirri presented at the anal opening. Three pairs post anal and one pair pre-anal papillae seen. From the above-mentioned cyst or bursa the skin is raised 5μ, all the way to the tip of the worm. No other peculiarity, and this may be only the usual male sexual end, such as I never saw before.

Locality—New York Aquarium.

Camallanus chelydrae

On March 17, 1915, there were collected eighteen *Camallanus* worms from the intestine of a *Chelydra serpentina.* There is no appreciable difference between these and the general description. They were all females and the intestine is filled with black material, partially digested blood.

Locality—New York Aquarium.

Camallanus floridianae, nov. sp.

(Fig. 62)

On June 20, 1916, on examining a Florida diamond-backed terrapin, *Chrysemys floridiana,* there were found in the stomach a number of Camallani, which, because of some difference between them and the other members of the genus, I propose the name *Camallanus floridianae.* There were collected and mounted three females and two males. The mouth has a different arrangement of the rods or knives. In most of these worms these rods are placed on two levels, one set of four being in front and the other set of four placed behind, and diagonally across the first. Whether this condition is only an exemplification of

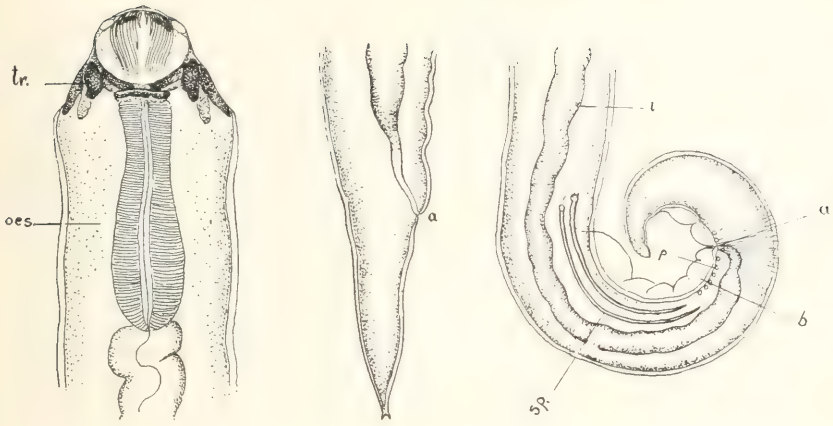

FIG. 61. *CAMALLANUS SCABRAE.*

FIG. 62. *CAMALLANUS FLORIDIANAE.*

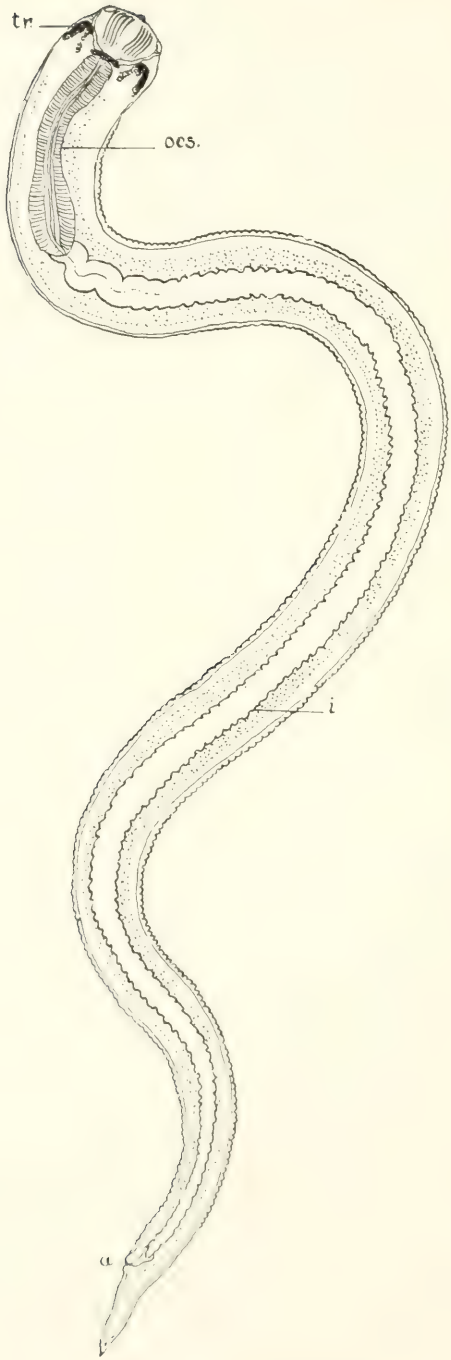

FIG. 63. *CAMALLANUS PTYCHOZOONDIS.*

the way in which they act normally by a sawing motion, I do not know but, this would look reasonable. They may act like a pair of hair clippers and were caught in this position when death took place. The mouth is, if anything, more concave than usual and there appear to be only two shoulder straps of chitin on each side of the neck instead of three, and in that case they would cease to be "tridents." They are not teeth anyway, Fig. 62. The males are uncoiled so that a sketch of that portion of the body was possible and revealed the fact that there are three post anal and six pre-anal papillae. They were all very long, especially the pre-anal ones, which are 7μ long and each with a knob at the end. The cirri were white and seen protruding a short distance. See bursa also Fig. 62. The larvae were not so plentiful, but seemed large, measuring 20μ long and 2μ wide. Seemed larger than in other worms.

Female—15 mm. long.
Male—7 mm. long.
Locality—New York Aquarium. Florida.

Camallanus elegans, sp. nov.

These worms were found in and collected from the stomach of Cumberland turtle, *Chrysemys elegans.* They were a lot of small red worms and several were mounted, 3 females and 3 males on one slide, and 4 females and 2 males on another. They measure about 15 mm. and 7 mm. respectively and the only difference to be seen from the general description is that the males which in two instances are uncoiled show a formidable looking single cirrus, which is thick and sharp, and the whole length is 90μ with about 7μ protruding in one case and 30μ in the other. The papillae are the same in number, but of a different shape from those described for *C. floridianae.* In this worm they are lower and thicker. This is the only cirrus I have seen protruded and while I thought that I could see the points of the two at the outlet in others, still I may have been mistaken, although I think not for they could be seen in the body. The bursal arrangement in this worm is quite different from any other. There is a sac not so divided and the whole affair is not so complete and pretentious as in *C. floridianae.* The host was kindly sent from the New York Aquarium and came originally from Florida. I propose the name *C. elegans* for it.

Camallanus ptychozoondis, sp. nov.

(Fig. 63)

On May 24, 1916, there were found in the rectum of a *Ptychozoon hemalocephalum* a leaf-like lizard taken near Buitenzorg, Java, a few *Oxyuridae,* and one small *Camallanus.* A description of the latter

follows. It is provided with a head and mouth, in shape somewhat like the concave side of a scallop shell. This part of it is yellow or brownish chitinous material concentrating posteriorly into a throat which is the beginning of the pharynx. This latter organ is flask-like in shape, so common in this genus, and is quite muscular. The intestine follows and finds its outlet on the left side of the worm near the end of the tail which is pointed and chitinous.

The vagina has not been seen, but appears to be very near the mouth on the left side and from this extends to the uterus, which is tubular and contains numerous round eggs. The worm is only about 5 mm. long and .20 mm. wide.

Camallanus cyathocephalus, sp. nov.

(Fig. 64)

Host—*Chrysemys scabra.*
Habitat—Stomach.
Locality—Zoological Park, New York.

While examining a slider terrapin, *Chrysemys scabra,* among some other *Camallanus* worms, I found one quite different from the others, as will be seen by a reference to the plate.

It is an immature female, that is, it has no eggs in its uterus, yet otherwise it is perfect. The head is the peculiar feature, since it is different from the ordinary *Camallanus,* having no "tridents" or adornments on the outside of the head. The whole structure gives the impression of a likeness to a wine-glass or goblet. The mouth is like that of the genus as far as shape and incising rods are concerned, yet it is vastly more delicate and proves a difficult task for an artist to portray and do it justice. It is composed of the usual amber-colored chitin. The worm is only 5 mm. long. The tail is trifid.

Camallanus bungari, sp. nov.

(Fig. 65)

On May 26, 1916, there were found in a Krait, *Bungarus candidus,* at Buitenzorg, Java, a small *Camallanus,* which probably hitherto has not been recorded. It has a wide open mouth as usual in the genus, which is sub-terminal, the real mouth, however, seems to be a circular opening near the center of the anterior margin of the round, amber-colored, chitinous structure, which lies open at the anterior end of the worm. From the small circular mouth, a tube, the oesophagus, leads into the pharynx. The open circular chitinous structure has within its outer margin two circular rings, which concentrate at the opening of the pharynx. The outer one of these is armed with very small spi-

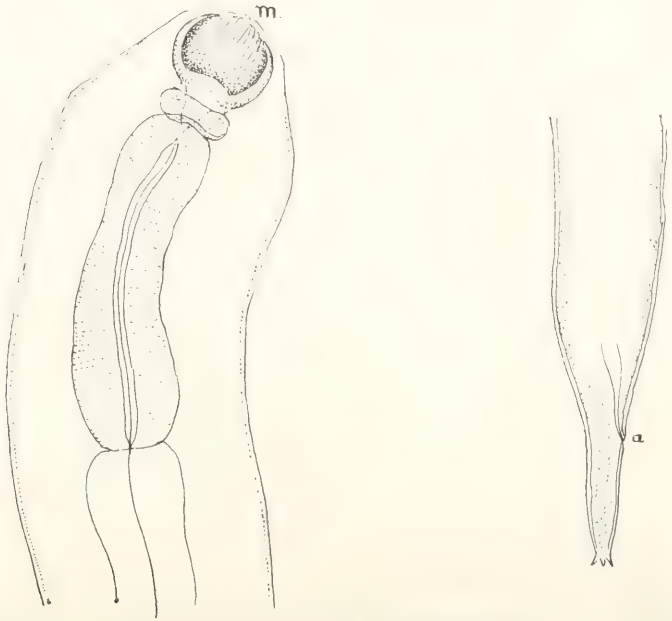

FIG. 64. *CAMALLANUS CYATHOCEPHALUS.*

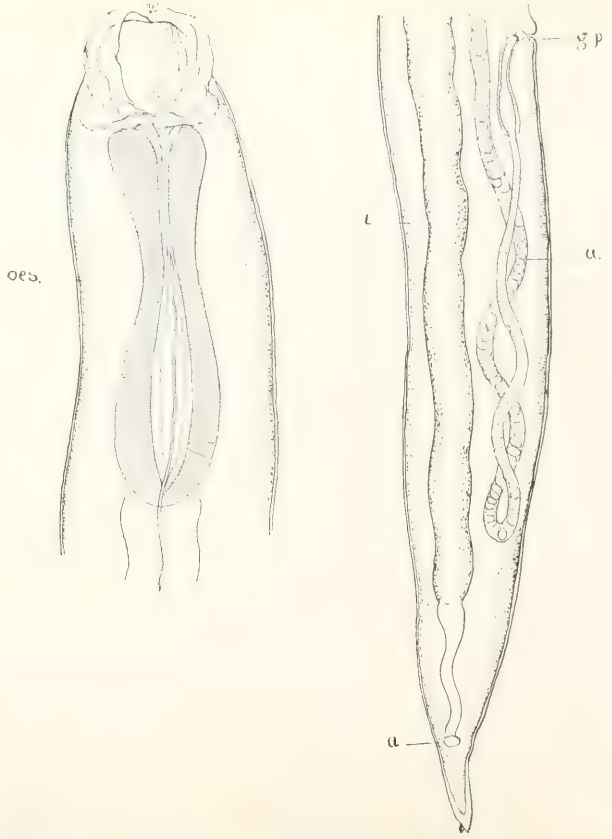

FIG. 65. *CAMALLANUS BUNGARI.*

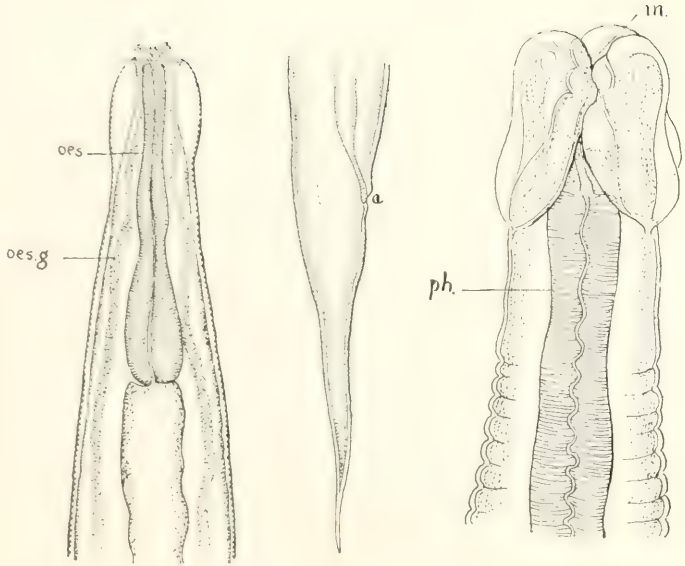

FIG. 66. *STRONGYLUS SIMIAE.*

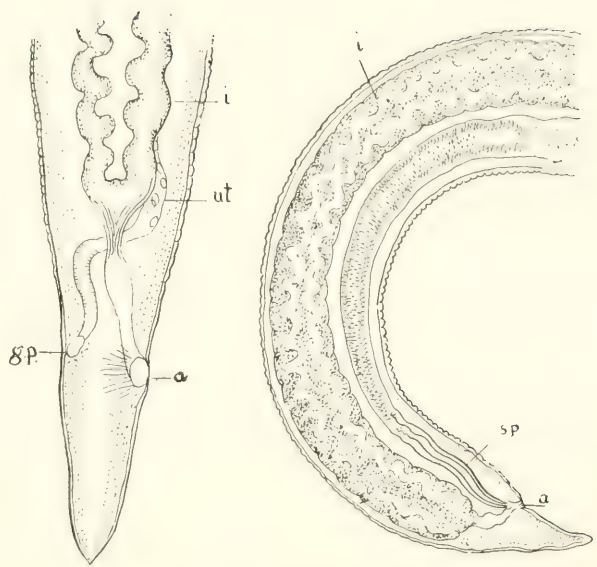

FIG. 67. *ASCARIS OGCOCEPHALI.*

cules or teeth, and the more or less circular anterior margin of the pharynx is also armed with small teeth. The pharynx is as usual flask-shaped and quite muscular. It terminates in the intestine and this latter has its outlet, in this case, near the posterior spiked tail. It is next to impossible to follow the windings of the genital apparatus in the body of this female worm, but it is almost certain that the genital opening is at the junction of middle and posterior thirds of the body. No other opening is seen. There is a tube which can be traced nearly to the anterior end of the worm, but it is undecided whether a vagina may be there. Tail trifid, while only three rods can be made out in this species, there is also a membrane which encircles the mouth being attached to its margin all around.

Strongylus simiae

(Fig. 66)

Host—*Simia satyrus.*
Habitat—Colon.
Locality—Singapore.

On July 21, 1916, while examining one of these animals, Dr. W. G. MacCallum came across several small nematodes in the colon, and saved them. They consist chiefly of Oxyurides, probably *Oxyuris vermicularis bremser* (*Ascaris vermicularis* Lin.), Diesing Syst. Helm. 11, p. 144-145. These worms are quite small, 6 or 7 mm. long by .40 wide and very sharp or pointed at each end, body filled with small eggs. Among these, however, I discovered three or four other worms which are strongyles and may be *Strongylus longivaginatus*, Diesing Syst. Helm, 11, p. 877-878 ventric. The only other strongyle known for this animal, according to Von Linstow, is *Strongylus duodenalis* Dubini, *Uncinaria duodenalis* Railliet, 1885, the same as in man. Of course, these are well known, but *S. longivaginatus,* I am not familiar with.

These strongyles, a figure of which is given, are small, all four females and filled with eggs. They measure 20 mm. long by .90 mm. wide. The body is prolonged into a very sharp tail and the skin is striated transversely throughout, although the striations are very delicate and hardly perceptible. The skin is also armed with fine denticles throughout, all pointing backwards. The head is 10μ across, but immediately behind the mouth the skin on each side spreads out so that it measures across this part 20μ. The mouth is armed with six small hooks. These with the enlargement behind the head enables the worms to retain its hold, when the head is buried beneath the mucous membrane of its host. The mouth is followed by a relatively long oesophagus, which is very muscular and is the sucking apparatus. On each side of the oesophagus, there is an oesophageal gland, or tube, which is said to convey poisonous matter into the tissues of the host. The

anus is situated about 200μ from the tip of the tail. The vulva is at the junction of the anterior and middle thirds of the length of the worm. Eggs 5μ long and 2μ wide. No males seen.

Ascaris ogcocephali, sp. nov.

(Figs. 67 and 67a)

On examining a bat-fish, Diabolo, *Ogcocephalus radiatus* (Mitchill, 1818), on April 18, 1917, from Key West, Florida, there were found a large number of ascarides in the small intestine and stomach.

The female worm is much larger than the male, and it is yellowish in color, while the male is quite small and whitish. They both are quite pointed at the extremities, especially the posterior, and the tail of the male is incurved. At the anus, which is very near the tip, may be seen three pairs of papillae post-anal and two pairs pre-anal. The cirri were not seen protruding, but could be seen internally. Along the dorsal portion of the abdominal cavity, there were a number of conical masses, which extend in two rows from a point near the middle of the worm to the posterior end.* The intestine, filled with dark-colored matter, extends from the oesophagus along the middle of the body to the anus. The oesophagus is long and muscular, and at its junction with the intestines may be seen two long coeca, one pointing posteriorly and the other recurved anteriorly. The head end is unlike that of any of the other ascarids. While there are the usual three lips or jaws, they are of a different shape and are furnished with lobulations of the skin, but unarmed. The neck is smooth for a short distance, then the skin becomes deeply striated transversely. The tail end of the female is straight and sharp, and a short distance from the tip on the one side is the anus, and on the other a little anterior is the genital pore. The uterus is much coiled and the eggs are numerous.

Measurements

Female length	32.00 mm.
Female width	.60 mm.
Male length	20.00 mm.
Male width	.35 mm.

Echinorhynchus gaboes, sp. nov.

(Fig. 68)

The *Echinorhynchus* belongs to the family of *Acanthocephala,* a branch of Nemathelminthes, or round worms, and are characterized by the possession at the head end of an armed proboscis, which they have

*These rows proved to be the walls of the intestine.

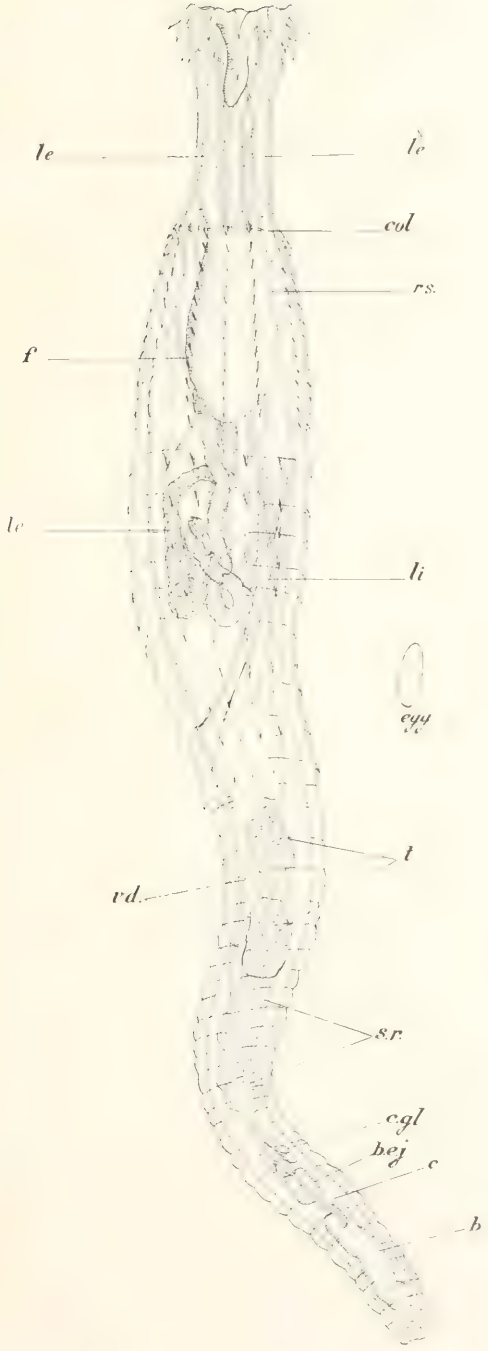

FIG. 68. *ECHINORHYNCHUS GABOES.*

the power of protruding or retracting at will They are not provided with a mouth or digestive system, their nourishment taking place through a process of osmosis. There is also a system of canals beneath the skin, which contain a lymph-like fluid, which is absorbed from their fluid surroundings, and there is also a pair of elongated tubes or organs near the head called the lemnisci which are credited with being the chief excretory organs. They are unusual in *E. gaboes*, since they are in a considerable coil posterior to the end of the proboscis sac. The skin is usually wrinkled and coarsely striated, and sometimes covered with strong spines, although this last feature is not common with the echino-rhynchi. There is one strong ligament running longitudinally, which seems to influence the main movements of the body. There is a very simple nervous system, which consists of a single ganglion situated at the base of the receiving sac of the proboscis, and from this nervous filaments radiate to different parts of the body.

They are of separate sexes, the male being smaller than the female The male is provided with two testes, several cement glands, a ductus ejaculatorius, and a cirrus which protrudes into a bursal sac, situated at the posterior end of the body, which in conjugation becomes everted. These are all the organs of generation in the male mentioned by authors, but in the case of *E. gaboes* there is interposed between the testes and the cement glands a large receptaculum seminis, which communicates with the ductus ejaculatorius by means of a duct. Fig. 68. The female is provided with an ovary and there appear to be a short vagina and a uterus ordinarily. The ova are developed in the ovary, which Claus and Sedgewick say subdivides into numerous masses of eggs. These, as they become ripe, burst and escape into the body cavity, and pass from this into the uterus, from which they are forced externally. The eggs are fusiform in shape, and generally in immense numbers.

Echinorhynchs are usually parasites of fishes or birds, chiefly the former. Only one is known as a parasite of man, *E. gigas* (Goeze, 1782). The present form, *E. gaboes*, was collected by Dr. W. G. MacCallum in Borneo from the intestine of a river fish named "Ikan gaboes," and, as will be seen from the figure, is an unusually armed form. The host of this Echinorhynch was obtained at Bandjermasin Borneo, and was a large fish, black above and spotted on whitish ground below. It was thought to be a sort of Siluroid fish, although it had large scales. I am unable to describe the internal structure of the female from the fact that from the neck to the posterior end of the body cavity is filled with immense numbers of large fusiform eggs, which cover over everything and are apparently loose in the cavity. They are 10μ long by 5μ wide and are in different stages of segmentation. The vulva is situated about 130μ from the posterior end. The male is 7 mm. long and the female 11 mm. and 70μ wide. The proboscis is 45μ long by 25μ wide, being wider at the end than in the middle. Some of the larger hooks on the proboscis are 7μ long and very strong.

On account of the peculiarities mentioned, I am calling this a new species. The internal structure of the male is unusual in the presence of a large seminal receptacle situated between the testes and the cement glands. The bursa is relatively large. The usual large ligament in this case is not in one single mass, extending from the base of the proboscis sheath to the bulbus ejaculatorius below, but is an open mass of filaments extending from the back of the proboscis sheath and proceeding posteriorly is spread out throughout the body as far back as the anterior testes, some of the filaments going even to the outer layer of the thick skin in some places. The skin is tranversely striated into coarse divisions and is strongly armed with large spines from the head to two-thirds the length of the body. They are all pointed backwards.

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Notes on the genus Microcotyle.

III.

By

G. A. MacCallum, M. D.

(From the Department of Pathology, College of Physicians & Surgeons, Columbia University, New York.)

With 3 figures in the text.

M. centropristes n. sp.

This form is found in very limited numbers upon the gills of the sea bass (*Centropristes striatus*), never, so far as observed, being abundant enough to affect the welfare of the host.

The worm is short and thick with a short stubby sucker disc supporting only thirty-five or forty-five suckers which have a chitinous armature quite similar to that of *M. angelichthys*, although much more heavily built and with rather longer lateral spines. These suckers are all quite alike in form and of about the same size.

The mouth is peculiar in that the lips are corrugated and the orifice subterminal. The mouth suckers are relatively large and round, and along the anterior margin there is a row of minute teeth. There is a partition which is often rather indistinct. In some specimens there can be seen a clear sac-like space behind each sucker as though there were a coecum connected with the sucker. The pharynx is muscular and is followed by a rather long oesophagus which divides into the intestinal rami.

The genital cloaca is bowl-shaped, the orifice being directed rather backward. It is lined by many fine sharply pointed spines directed inward and upward and numbering at least eighty: behind these there are two small groups of similar spines. The vas deferens and the uterus may be traced into this bowl-shaped opening. The vagina is very plainly seen as a single median tube leading from a dorsal opening to divide into the two lateral ducts of the vitellarium which then reunite posteriorly to form the vitellarium reservoir. The ovary is very indistinctly outlined. The eggs are thick-walled and deep yellow. They have only very short spur-like processes at each end with no filament, or are rounded at the ends.

There are eight or ten rather large testes. The vas deferens terminates in a rather pyramidal or conical mass in the interior of the cloaca which is bordered by the two small accessory clusters of spines and may represent the cirrus.

Measurements.

Length	3 mm
Width	0,6
Sucker disc	1,0 × 0,5
Suckers	38—45
Testes	8—10
Eggs	0,15 mm in length
No filament	
Diam. of sucker disc	0,80 × 0,60

New York Fish Markets.

M. poronoti n. sp.

The are found pretty regularly moderate numbers of this species clinging to the gills of the Butterfish (*Poronotus triacanthus*) which were brought to the U. S. Fish Commission Laboratories at Woods Hole.

The worm when at rest is about 6 mm long by 1 mm at its widest part. There is a long caudal sucker disc armed with one hundred and twenty suckers which forms about one-third of the whole length of the worm. The suckers are flattened laterally: their chitinous skeleton is in general like that of *M. centropristis* but its lateral spur is very short. In one specimen they are seen from the edge and it is plain that the ends of the main arches are

sharply bent so as to form teeth which give the sucker a better hold. In the anterior lip there are three clumps of cell-like palely stained bodies probably representing sense organs.

The neck is long and graceful. The genital cloaca is oval with a small round anterior opening. The short triangular spines, of which there are a great many, seem to be distributed pretty evenly over the outside of this sac-like structure but are largest and most closely placed about the orifice. Evidently it is a somewhat eversible sac. Behind it there is seen an olive shaped mass which is the termination of the rather muscular vas deferens and which must therefore represent the cirrus. It is flanked on either side by a group of about fifteen spines and in front of it there is apparently a separate opening.

The ovary is placed more or less across the middle of the worm and is not very large. The oviduct can be plainly seen to be joined by the neck of the seminal reservoir which lies posterior to it on the left side of the worm, and by the vitelline duct after which it passes into the shell gland and the uterus. The eggs occur in small number, usually one or two only, and are provided with rather short stout prolongations at each end. There is no long finely coiled filament as in some other forms.

There are thirty-two rather large testes from which the somewhat thickwalled vas deferens runs forward.

Measurements.

Length	6 mm
Width	0,80
Sucker disc	2,50
Number of suckers	120
Diameter	0,05
Number of testes	32
Eggs	0,31 × 0,04
Anterior filament	0,24
Posterior filament	0,07

U. S. Fish Commission. Woods Hole.

M. pomocanthi n. sp.

In describing *M. angelichthys* in a previous paper it was stated that in its structure that worm resembled in many points others to

be described later, and it is on account of the close resemblance of at least six of these forms that we have hesitated to publish their description until it could be more definitely ascertained whether they really belong to one species or to several. *M. angelichthys* differed from all of these six worms in the possession of a globular ovary and was therefore set down as a distinct species.

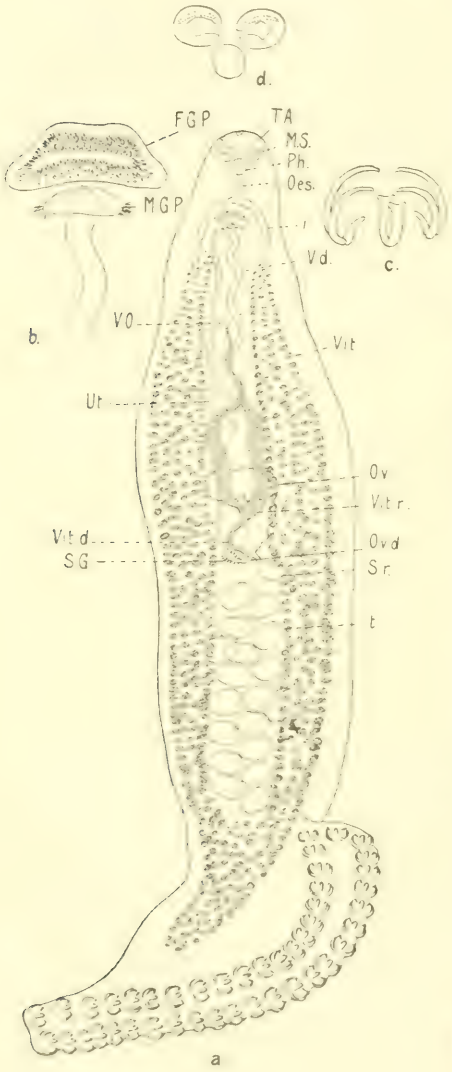

Fig. A.

C Cirrus
C. d caudal disc
F. G. P female genital pore
G. P genital pore
i intestine
M mouth
M. G. P male genital pore
M. S mouth sucker
Oes oesophagus
OOt ootype
Ova egg
Ov ovarium
Ov. d oviduct
Ph pharynx
S. C caudal disc sucker
S. G shell gland
S. r seminal reservoir
t Testes
T. A Tactile area
Ut uterus
Va vagina
V. d Vas deferens
Vit vitellaria
Vit. d Vitelline duct
Vit. r Vitelline reservoir
V. O vaginal opening

Fig. A.

a *Microcotyle centropristes n. sp.*
b Male and female genital pores.
c Chitinous frame of a caudal sucker.
d Mouth sucker.

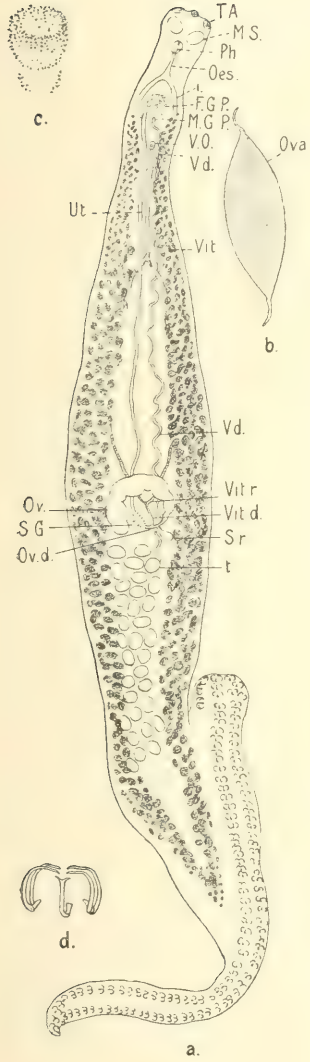

Fig. B.

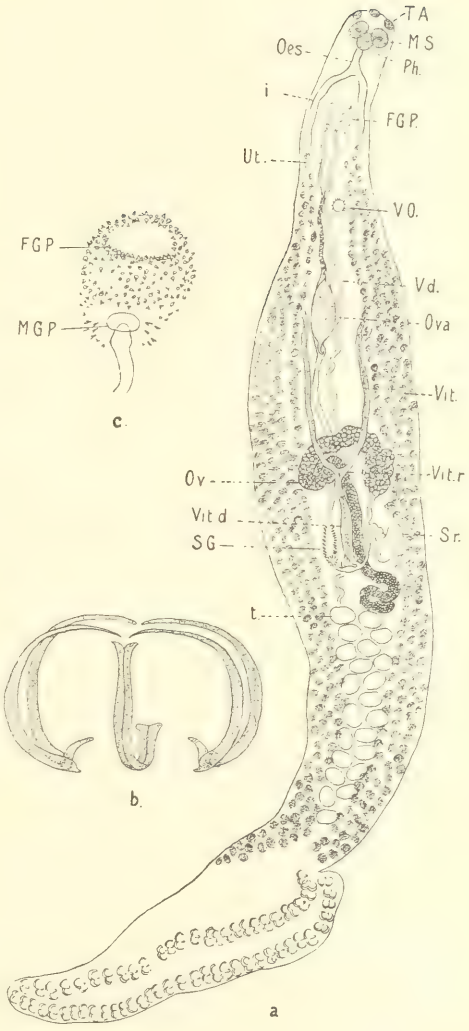

Fig. C.

Fig. B. a *Microcotyle poronoti.* b Ovum. c Female genital pore. d Chitinous skeleton of a caudal sucker.

Fig. C. a *Microcotyle pomocanthi.* b Chitinous skeleton of a caudal sucker. c Genital pores, male and female.

In the course of examining many fish of various sorts, worms belonging to the genus *Microcotyle* were found attached to the gills of the Black angel fish (*Pomocanthus arcuatus*), the Butterfly fish (*Chaetodon ocellatus*), the small Butterfly fish (*Chaetodon capistratus*), the Grass porgy (*Calamus arctifrons*), the Pork fish (*Anisotremus virginicus*), the Yellow finned grouper (*Epinephalus flavolimbatus*), and the Spanish hogfish (*Harpe rufa*). These are so much alike that I hesitate to describe them as separate species and even in describing them under one name would point out the remarkable similarities which exist between them and *M. caudata*, *sebastes*, *elegans* and even *stenotomi* of Goto. It is true that these four species of Goto differ among themselves slightly in size, in the number of suckers and in the number of testes but otherwise they are remarkably alike. Furthermore it is clear from the study of immature forms that the size, number of suckers and number of testes increase with age. Naturally one might suppose that if the worms are quite mature a constant number would be attained but it is common enough to find worms of various sizes and ages upon the gills of the same fish and difficult to decide which of these is quite mature. Therefore such numbers seem hardly acceptable as criteria of specific differences and are not to be compared in importance with the marked differences in form of the suckers, genital armature etc. which form the basis for most cases of specific differentiation.

For that reason it is with the idea of a later and more careful revision of this whole group and because the forms do not correspond very closely with Goto's that they are described together under one specific name.

All of these worms are about 3,5—4,5 mm $\times$ 0,6—0,7 mm in size with a sucker disc which curves up backward and is furnished with fifty to seventy-five suckers ranged along the ventral margin and projecting laterally on short stalks. These suckers have a rather delicate chitinous skeleton of the form shown in the drawing with short lateral spur and are uniform in character.

The mouth suckers are unarmed and obliquely placed at the sides of the mouth. They are divided by a partition. The mouth is slightly subterminal with an overhanging lip. The pharynx is well developed and leads into a rather short oesophagus which bifurcates into the lateral intestinal coeca.

Just behind the bifurcation is the armed genital opening. In

all the worms this presents the appearance seen in the drawing. — an oval or nearly spherical bulbous mass covered over with minute spines which point backward and extend into the eversible small orifice which forms the outlet for the uterus. Behind this there is a second aperture for the small conical cirrus-like end of the vas deferens. This is guarded on each side by an elongated group of about ten or twelve similar short spines.

The ovary is a somewhat indefinite elongated sac crossing the middle of the body and extending backward. — Its oviduct receives the channel from the clearly defined seminal reservoir and that from the vitelline duct and passes through the shell gland into the straight uterus.

The vaginal orifice is median and dorsal and the vagina divides some distance behind this to join the vitelline ducts which run back laterally receiving branches from the vitellarium to reunite in forming the vitelline reservoir which sends its duct to join the oviduct.

The eggs, one or two in number, are provided posteriorly with a rather stout tapering prolongation, anteriorly with a similar structure which is elongated, however, into a very long and delicate tangled and coiled filament. The testes vary in number from eighteen to twentyseven and send as usual the undulating vas deferens through the mid line of the body forward to the genital opening.

Measurements.

Length	3,5—4,5 mm
Width	0,6—0,7
Length of sucker disc	7—1,0
Suckers	50—78
Size of Suckers	0,30×0,2
Eggs	0,12×0,08
Testes	18—27

New York Aquarium.

Many of the fish belonging to the families Chaetodontidae, Sparidae and Haemulidae which I have obtained from the New York Aquarium have been found infected with the *Microcotyle* just described. So severe is this infection that it may be said to have been the cause of death in many instances especially in the Chaetodontidae where it is almost universal.

As I have said in a former paper, certain worms seem to be limited to particular families of fish but it is impossible to say whether fishes in the wild state are as badly infested as those in confinement. It may be that the fish in this group belonging to different genera acquire their infestation with this particular worm from infected tanks even though all ordinary precautions are observed to keep them clean — a point which we shall attempt to clear up.

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THE EFFECTS OF INTRAVENOUS INJECTIONS OF DICHLOROETHYLSULFIDE IN RABBITS, WITH SPECIAL REFERENCE TO ITS LEUCOTOXIC ACTION.*

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Plate 10.

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INTRODUCTION.

There exists evidence pointing towards the general toxicity of dichloroethylsulfide both when administered by inhalation and when injected subcutaneously or intravenously.

Lynch[1] found that dogs gassed with high concentrations of dichloroethylsulfide (0.3 mg. per liter for 1 hour) exhibited symptoms similar to those produced by injection, and not referable to the primary irritation of the respiratory tract. These symptoms were salivation, vomiting, bloody diarrhea, hyperexcitability, and convulsions, with a slow irregular pulse which became rapid before death, and were attributed to vagal paralysis. Furthermore, the absorption of dichloroethylsulfide during inhalation was shown by the appearance of the hydrolysis product, dihydroxyethylsulfide, in the urine.

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* The experiments were performed at the Experimental Field of the Chemical Warfare Service, American Expeditionary Forces, and were continued by one of us at the Brady Laboratory of Pathology of the Yale Medical School.

We are indebted to Lieutenant Colonel H. C. Clark, Chemical Warfare Service, for advice, to Professor M. C. Winternitz for the privilege of his laboratory, and to Professor F. P. Underhill for procuring samples of nitrobenzene and chlorobenzene.

A preliminary note of this work was presented at the meeting of the Society of Experimental Biology and Medicine, New York, March 19, 1919.

[1] Lynch, V., American University Experiment Station, Monograph No. 1, Washington, 2nd edition, 1918, 317. See also Lynch, V., Smith, H. W., and Marshall, E. K., Jr., Dichlorethylsulphide (mustard gas). I. Systemic effects and mechanism of action, *J. Pharmacol. and Exp. Therap.*, 1918–19, xii, 265.

Severe symptoms, ending usually in death within 24 hours, followed the intravenous injection of approximately 0.01 gm. per kilo in dogs. At autopsy intense congestion, often with extensive hemorrhage into the intestinal mucosa, was the only lesion noted.[2]

Muratet and Fauré-Fremiet[3] reported upon blood examinations in a series of six rabbits, four of which were poisoned by inhalation and two by subcutaneous injection. In all the animals the following changes were noted. The red blood cells were augmented in number soon after exposure (increase to 6,000,000 to 10,000,000), and then gradually diminished. Nucleated forms were sometimes seen. There was no polychromatophilia or other degenerative change. The leucocytes showed a rise following the intoxicating dose, but later diminished progressively. There was a relative lymphocytosis. Morphologically, degenerative changes were found in the leucocytes. The polymorphonuclears showed abnormal lobulation of the nucleus, dissolution of the granules, and, finally, a breaking up of the chromatin into spherical masses. The lymphocytes also showed degenerative changes, and many abnormal cells which could not be identified were present in the films. Jolly[4] repeated the experiments of Muratet and Fauré-Fremiet, using both dogs and rabbits, and administering the dichloroethylsulfide both by inhalation and subcutaneous injection. His results were less consistent than those of the previous workers, but a study of the figures obtained seems to show a rather constant diminution of leucocytes after injection; with inhalation the results were variable.

Zunz[5] observed in severely gassed human cases a leucopenia with relative lymphocytosis developing after several days.

Stewart,[6] in a study of the blood changes in six fatal cases of poisoning with mustard gas, found an initial polymorphonuclear leucocytosis, followed by a rapid fall in the total and neutrophil count, beginning on the 3rd or 4th day, and reaching an extremely low figure (200 to 500) before death. The leucopenia is ascribed to acute failure of the leucoblastic function, without associated erythroblastic failure. No histological study of the bone marrow is included in the report.

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[2] Winternitz, M. C., Finney, W. P., and Wislocki, G. B., American University Experiment Station, Monograph No. 1, Washington, 2nd edition, 1918, 389.

[3] Muratet, L., and Fauré-Fremiet, Confidential Report of the French Gas Service.

[4] Jolly, Confidential Report of the French Gas Service.

[5] Zunz, Report to the Interallied Gas Conference, Paris, 1918.

[6] Stewart, M. J., Report on cases of poisoning by "mustard gas" (dichlorethylsulphide) with special reference to the histological changes and to alterations in the leucocyte count, Report of the Chemical Warfare Committee, *Great Britain Med. Research Com. Rep., No. 17*, 1918.

Krumbhaar[7] also reported a series of human mustard gas cases in whom there developed, after the initial leucocytosis, a marked leucopenia, persisting even in the presence of the complicating bronchopneumonia. Moderate anemia, without blast formation, and an apparent decrease in the number of platelets were also observed. Postmortem examination of the bone marrow showed a "greater or less disappearance of normoblasts, myelocytes and adult forms," only "primordial cells and megaloblasts" remaining. Krumbhaar's observations offer convincing evidence of a systemic effect in severe cases of mustard gas inhalation. It is highly improbable that the leucopenia and other blood changes were due to complicating influenzal infections, inasmuch as practically all these gas cases occurred previous to the development of the influenza epidemic.

Hermann,[8] in his study of the clinical pathology of mustard gas poisoning, finds "no leucopenia at any time," the changes observed being slight secondary anemia with a well marked polymorphonuclear leucocytosis, a definite eosinophilia, and the appearance of myelocytes and young forms of leucocytes. The value of Hermann's observations is lessened by the fact that in none of the eight cases upon which his report of the blood changes is based, was the blood examined before the 10th day after gassing, and that only one of his cases was fatal, and the majority mild in character.

Warthin and Weller[9] studied the effects of subcutaneous and intravenous injection of dichloroethylsulfide in a series of rabbits and dogs. The toxic symptoms described are salivation, diarrhea, depression of temperature, and increased rapidity of respiration followed by slowing. With large doses animals, after a short period of nervous excitement, die within a few hours in coma with gradual failure of respiration. The gross lesions, apart from the local effect, consist of a general passive congestion of all organs with minute hemorrhages, emboli, and infarctions. The only specific changes are more or less severe catarrhal enteritis with corresponding microscopic findings. A striking microscopic feature is the presence of hemorrhages and pigmented phagocytes in great numbers in the splenic tissue. Intravenous injections (dose of 0.0075 to 0.18 cc.) are followed, after a quiescent period, by general convulsions, opisthotonos, irregular jerking movements, salivation and lacrimation, and fall of temperature. The animal passes into coma, and there is gradual respiratory and cardiac failure. Patho-

[7] Krumbhaar, E. B., Rôle of the blood and the bone marrow in certain forms of gas poisoning. I. Peripheral blood changes and their significance, *J. Am. Med. Assn.*, 1919, lxxii, 39.

[8] Hermann, G. R., The clinical pathology of mustard gas (dichlorethylsulphide) poisoning, *J. Lab. and Clin. Med.*, 1918–19, iv, 1.

[9] Warthin, A. S., and Weller, C. V., The general pathology of mustard gas (dichlorethylsulphide) poisoning, *J. Lab. and Clin. Med.*, 1919, iv, 265; also in Researches on the pathology of mustard gas (dichlorethylsulphide) poisoning, Contributions from the Pathological Laboratory, University of Michigan, 1918–19, ix.

logically, there are found dilatation of the right side of the heart, congestion, petechia, hemorrhages of the lungs, and general congestion of other viscera. We have found no reference in Warthin and Weller's papers as to changes in the circulating leucocytes or bone marrow except the general statement[10] that animals exposed to direct application in the gassing chamber may show, among other effects, "secondary anemia, leucocytosis or leucopenia."

The following report deals with the effects of the intravenous injection of dichloroethylsulfide in rabbits. We have given special attention to the alterations in the blood picture and in the blood-forming organs, since these were the most striking of the results observed.

Technique.

Method of Injection.—Difficulty was at first experienced in obtaining suitable emulsions for injection. In the earlier experiments the given amount of a 10 per cent alcoholic solution by weight of dichloroethylsulfide was suspended in 0.85 per cent salt solution, shaken vigorously, and immediately injected, after allowing the larger globules to settle out. The dose administered was, therefore, considerably less than the total amount taken. Later it was found that a more satisfactory suspension could be made by emulsifying in 30 per cent alcohol in distilled water. A slightly milky emulsion was obtained which after shaking did not separate out in the time necessary for injection. The suspension was prepared from a recently made, accurately weighed 10 per cent solution in absolute alcohol, and immediately injected to avoid hydrolysis. The dichloroethylsulfide used was a distillate from the contents of a German yellow cross shell and was actively vesicant.

Dose.—We have not attempted to determine accurately the minimum lethal dose. It was found in the earlier experiments that the limit of tolerance was in the neighborhood of 0.01 gm. per kilo, and in the last six rabbits used a uniform dose of 0.005 gm. per kilo was chosen.

[10] Warthin, A. S., and Weller, C. V., The general pathology of mustard gas (dichlorethylsulphide) poisoning, *J. Lab. and Clin. Med.*, 1919, iv, 266.

Symptoms.

Emaciation and Loss of Weight.—These symptoms were noted in all the rabbits which survived for 2 days or more after the injection. In all but one animal (Rabbit 1, loss of weight only 100 gm.) this was associated with diarrhea, and perhaps was due to it.

Nervous Symptoms.—These were observed only in Rabbits 2 to 4, which died 1 hour, 1½ hours, and during the night following injection. The animals showed extreme restlessness, incoordinate movements, retraction of the head, and transient spasticity, but no definite paralyses or convulsions.

Respiratory Symptoms.—No definite or characteristic respiratory symptoms were observed even in animals dying a few hours after the injection, in which the occurrence of pulmonary embolism might have been suspected.

Intestinal Disturbances.—Diarrhea occurred in six animals, in all but one associated with the finding of gross lesions of the intestinal tract at autopsy. The feces were copious, fluid, dark brown, and not grossly admixed with blood or mucus.

Edema.—Edema of the ears of wide extent invariably followed injection, even when great care was taken to avoid introducing the material outside the vein. It would appear that the dichloroethylsulfide diffused readily through the vessel wall. It was also observed that after the injection was begun an area of blanching, involving the neighboring skin over a width of several centimeters, at once appeared. This persisted for a few minutes after the injection, after which the normal circulation was restored.

Pathology.

Respiratory Tract.—Of twelve rabbits injected, four showed definite pulmonary lesions (Nos. 2 to 5). All these animals died or were killed within a period of from 1 to 22 hours following the injection, and none of the eight animals surviving over 24 hours showed significant gross or microscopic lesions.

The changes observed were (1) irregular areas of edema, in part fibrinous; (2) areas of atelectasis and emphysema; and (3) accumula-

tions of leucocytes in the capillaries, often showing caryorrhexis and fragmentation, and slight emigration into the alveoli. The trachea and bronchi were normal except that they contained a homogeneous coagulum. No thrombi were found in the capillaries or larger vessels. The small pulmonary arteries were thick walled and appeared contracted. Clear vacuoles were seen beneath the endothelium. The significance of this finding is not clear as somewhat similar pictures may be seen in normal animals.

It is, of course, not possible to conclude from the histological findings that dichloroethylsulfide is eliminated by the pulmonary epithelium; on the other hand, no support is found for the view that the lesions are the result of capillary embolism due to impaction of dichloroethylsulfide globules. The fact that animals which survived for a longer period showed no pulmonary lesions might suggest that the edema is the result of a direct and immediate action of the dichloroethylsulfide upon the pulmonary capillaries.

Alimentary Tract.—No lesions were found in the esophagus. Rabbit 5 showed hemorrhages into the pyloric portion of the stomach and in the duodenum. Of the remaining rabbits, three (Nos. 6, 7, and 8) had a severe diphtheritic enteritis affecting the middle or lower portion of the intestine. Rabbit 6 showed also patches of membranous inflammation in the large intestine. Rabbit 9 also had a diphtheritic colitis, but this proved to be coccidial in origin. Seven rabbits were free from lesions of the intestinal tract.

Liver.—The liver showed no significant changes.

Nervous System.—No detailed study has been made. Neither capillary thrombosis nor hemorrhage was found.

Kidneys.—Definite changes were present only in the kidneys of Rabbits 2 and 5. The capsular spaces contained hyaline globules and occasionally red blood cells. Hyaline material was also present about the blood vessels in the intermediate zone between cortex and pyramids. Blood cells and hemoglobin (?) casts were found in the collecting tubules. The urine was not examined.

Blood and Hematopoietic System. General Considerations.—Since the observations of previous workers upon the blood changes following the administration of dichloroethylsulfide had yielded somewhat conflicting results, it was decided to undertake a detailed study of a

small series of rabbits, eliminating as far as possible incidental factors which might influence the blood picture. Of these incidental factors, variations in the surrounding temperature were found to be the most disturbing. When rabbits were exposed to a temperature of from 40–50°C. for ½ hour, the total leucocytic count showed a tendency to fall, although there were individual exceptions. Counts made shortly after removing the animals from the warm chamber to room temperature (15–20°C.) invariably showed an abrupt and striking rise (Table I). After this point had been established, the rabbits were kept in the laboratory during the observation period and following the injection in order to avoid sudden chilling or abrupt temperature change. Daily counts were made at approximately the same time (9 to 11 a.m.) before food was given. The differential counts are based upon an enumeration of 500 cells, except when the extreme leucopenia made this impracticable. In spite of the usual precautions unexplained variations occurred which made it difficult to draw conclusions as to the percentile fluctuation. In making the counts blood was always taken from the ear not used for injection. When both ears had been used, a small cut was made in the skin of the abdomen and blood taken from one of the superficial abdominal veins. At least two counts were made on each rabbit before injection.

Erythrocytes.—Stress of other work prevented a detailed study of the numerical variation in the erythrocytes. Preliminary observation (Rabbit 9) showed no significant change. There were no striking morphological changes pointing to a marked anemia, except, perhaps, the occurrence of a moderate anisocytosis in the terminal stages. Polychromatophilia is not infrequently seen in films from normal rabbits. Rabbit 13, during the period in which recovery from the effects of an injection was taking place, showed numerous normoblasts—an indication that the erythroblastic tissue had suffered injury, as well as the leucoblastic. Histological study of the bone marrow and spleen affords further evidence of the toxic action of dichloroethylsulfide upon the formation of red blood cells, which will be described.

Leucocytes.—In all but two rabbits (Nos. 8 and 12) of those surviving more than 24 hours, there occurred after a single injection of 0.005

to 0.01 gm. of dichloroethylsulfide per kilo a pronounced fall in the number of circulating leucocytes. In these two rabbits a second injection of the same dose after 7 and 8 days respectively was followed by the typical reaction. The leucopenia was preceded by a transient rise in only one rabbit (No. 7), but it should be noted that no counts were made at short periods following the injection. Although degenerated leucocytes with poorly staining and fragmented nuclei and vacuolated cytoplasm were occasionally found in films, they were infrequent, and even in the presence of an extreme leucopenia the rare leucocytes present in the films were usually normal morphologically. Blood platelets were found at all stages and showed no alterations. As regards the behavior of the different types of leucocytes, a study of these data shows that in some instances the injection is followed by an absolute and percentile increase in the polymorphonuclears, which fall rapidly with the onset of the leucopenia. In the terminal stages the polymorphonuclears practically disappear from the peripheral blood. On the other hand, sometimes an initial fall in the number and percentage of polymorphonuclears is followed by an absolute and relative increase. This secondary rise is associated with the appearance of unripe forms in considerable numbers (Rabbits 12 and 13), and coincides with regenerative activity of the bone marrow, as shown by a study of sections from rabbits killed at this stage. The leucopenia is accompanied by a relative lymphocytosis. The absolute number of lymphocytes is diminished in the later stages and lags behind that of the granular cells in the cases in which regeneration is occurring. There is a percentile increase in the large mononuclear cells, but their absolute number is unchanged or diminished.

The data showing the alterations in the blood count are presented in Table I and in Text-figs. 1 to 3.

Bone Marrow.—Although the appearance of the marrow varies in different animals, there is evidence of the destructive effect of the dichloroethylsulfide upon the blood-forming elements. The variations observed can be correlated with different stages of injury and repair, and these again are reflected more or less closely in the blood picture during life. The following brief descriptions will illustrate the different phases observed.

Rabbit 1 died 4 days after injection. At the time of death the leucocyte count had fallen to 800, of which 46 per cent were polymorphonuclears. The marrow of the femur contains a large amount of adipose tissue, the fat cells being separated by a loose edematous tissue containing less than the normal number of cells. The leucocytes of the granulocyte series are almost without exception degenerated. The cytoplasm in sections stained with Wright's stain (normal control) shows no granules. The nuclei stain diffusely and are frequently fragmented. The megacaryocytes also show degenerative changes. Some contain clumps of pink-staining, hyaline material in their cytoplasm. There are islands of apparently normal erythroblasts. The blood sinuses are wide and intensely congested with normal appearing red blood cells. They contain practically no nucleated elements. The appearances are interpreted as indicating an active injury to the bone marrow. The toxic action seems to have affected especially the granular cells.

Rabbit 8 died 4 days after a second injection of dichloroethylsulfide. Leucocytes on the last 2 days had fallen to 800, a film of the peripheral blood showing very few nucleated cells, which were almost exclusively large and small mononuclears. Sections of the marrow show an extreme aplasia, comparable with that seen in experimental benzene poisoning. Myelocytes, polymorphonuclears, and megacaryocytes have practically disappeared. There are loose collections of normoblasts scattered through the edematous fat tissue. Occasional globular fragments of chromatin, often enclosed in phagocytes, represent the remains of the destroyed cells. The sinuses are congested and contain no leucocytes (Fig. 1).

Rabbit 12 was killed on the 7th day following a second injection. The leucocytes which had fallen to 1,000 on the 3rd day after injection then rose to 1,400 and 2,100, and at the same time large numbers of myelocytes appeared in the peripheral blood. The histological picture shows the effect of a previous injury and at the same time an active regeneration. The predominant cell type is the myelocyte, the granules of which are definite and well stained in Wright's preparations. There are also a fair number of adult polymorphonuclears. The myelocytes are congregated in islands, as are also the erythroblasts. Megacaryocytes are numerous and are not

TABLE I.

Animal No.	Date.	Temperature.	Dose per kilo.	Red blood cells.	Hemoglobin.	Total No. of leucocytes.	Polymorphonuclears.		Lymphocytes.		Large mononuclears.		Other forms.
							Per cent.	No. per c.mm.	Per cent.	No. per c.mm.	Per cent.	No. per c.mm.	
	1918	°C.	*gm.*		*per cent*								
10	Aug. 20		0.005										
	" 23			5,280,000	90	2,300	3	70	76	1,748	21	490	
	" 24			6,000,000	90	1,000	3	30	86	860	11	110	
	" 25			4,960,000	90	1,000							
							Died.						
1	Sept. 9					8,800	9	790	85	7,480	6	528	
	" 10					7,500	25	1,880	67	5,030	8	600	
	" 12, 9.00 a.m.	13				7,000	49	3,430	43	3,010	8	560	
	10.35 "	40–56				5,900	32		62		6		
	11.30 "	18				10,400	63		31		6		
	" 13, 9.00 "	11				4,800	36	1,730	55	2,680	9	430	
	11.00 "	30–40				5,400	25		66		9		
	11.30 "	13				18,800	31		67		2		
	" 14, 9.00 "	13				4,900							
	10.30 "	50				4,900							
	11.30 "	16				6,600							
	" 15, 9.00 "	?				6,600							
	10.30 "					6,200							
	11.30 "					5,600							
	" 16, 2.00 p.m.					7,100							
	2.30 "		0.01										

	3.00 p.m.					4,600								
	Sept. 17, 9.00 a.m.					4,900								
	" 18					6,400								
	" 19					1,800	24	430	58	1,050	18	320		
	" 20, 10.30 a.m.			5,680,000	70	900	Practically all mononuclears.							
	3.00 p.m.					400								
							Died.							
4	Sept. 9					17,400	56	9,750	39	6,780	5	870		
	" 10					15,300	11	1,680	70	10,710	19	2,910		
	" 12, 9.00 a.m.	13				17,500	54	9,450	40	7,000	6	1,050		
	10.35 "	40				8,800	41		53		6			
	11.30 "	18				24,200	72		22		6			
	" 13, 9.00 "	11				8,000	43	3,440	50	4,000	7	560		
	10.30 "	30–40				13,600	68		25		7			
	11.00 "	13				20,000	52		42		6			
	" 14, 9.00 "	13				11,700								
	10.30 "	50				6,800								
	11.30 "	16				26,200								
	" 15, 9.00 "	17				12,400								
	10.30 "	Warm room (?).				19,200								
	11.30 "	19				10,400								
	" 16, 2.00 p.m.					12,300								
	2.30 "		0.01											
	3.00 p.m.					15,400								
	10.00 "					8,200								
							Died.							
6	Sept. 12, 9.00 a.m.	13				12,000	58	6,960	40	4,800	2	240		
	10.35 "	40–56				5,800	70		27		3			
	11.30 "	18				18,200	61		37		2			

Where the hour is not given the blood counts were made at 9 to 9.30 a.m.

TABLE I—*Continued.*

Animal No	Date.	Temperature.	Dose per kilo.	Red blood cells.	Hemoglobin.	Total No. of leucocytes.	Polymorphonuclears.		Lymphocytes.		Large mononuclears.		Other forms.
							Per cent.	No. per c.mm.	Per cent.	No. per c.mm.	Per cent.	No. per c.mm.	
	1918	*°C.*	*gm.*		*per cent*								
6	Sept. 13, 9.00 a.m.	11				12,000	62	7,440	34	4,080	4	480	
	10.30 "	30–40				12,800	55		35		10		
	11.30 "	13				13,800	66		31		3		
	" 14, 9.00 "	13				9,400							
	10.30 "	50				10,200							
	11.30 "	16				21,600							
	" 15, 9.00 "					11,600							
	10.30 "					8,400							
	11.30 "					14,100							
	" 16, 2.00 p.m.					10,200							
	2.30 "		0.01										
	3.00 p.m.					8,200							
	Sept. 17, 9.30 a.m.					10,200							
	" 18, 10.30 "					3,100	27	850	59	1,830	14	420	
	2.30 p.m.					1,700	7	120	83	1,410	10	170	
	" 19					1,100	10	110	69	760	21	230	
							Died.						
11	Sept. 21, a.m.					11,200	35	3,900	59	6,600	6	700	
	" 22, "					13,400	44	5,900	45	6,030	11	1,470	
	" 23, 9.00 a.m.			6,160,000	90	12,200							
	10.00 "		0.005										

		3.30 p.m.					9,100							
	Sept. 24						11,200	89	9,970	10	1,120	1	110	
	" 25						2,600	22	570	72	1,880	6	150	
	" 26						1,900	28	530	65	1,240	7	130	
	" 27						3,600	11	400	21	520	25	900	Myelocytes (?) 43 per cent.
	" 28							Died.						
7	Sept. 21						12,600	45	5,700	50	6,300	5	630	
	" 22,	9.00 a.m.	13				11,800	54	6,400	34	4,000	12	1,400	
		10.30 "	28				10,800							
		11.30 "	13				17,200							
	" 23,	9.00 "			5,680,000	90	16,400							
		10.00 "		0.005										
		3.00 p.m.					28,600							
	Sept. 24						13,800	95	13,100	5	700			
	" 25						3,800	80	3,040	19	720	1	40	
	" 26						800	46	368	44	352	10	80	
	" 27							Died.						
12	Sept. 21						11,200	62	6,940	37	4,140	1	120	
	" 22,	9.00 a.m.	13				10,000	82	8,200	16	1,600	2	200	
		10.30 "	28				11,600							
		11.30 "	13				20,600							
	" 23,	9.00 "			5,680,000	90	13,400							
		11.00 "		0.005										
		3.30 p.m.					13,200							
	Sept. 24						21,200	80	16,960	12	2,540	8	1,700	
	" 25						24,800	78	19,350	14	3,470	8	1,980	
	" 26						33,600	46	15,480	41	13,800	13	4,320	
	" 27						40,800	56	22,840	30	12,240	14	5,720	
	" 28						23,600	41	9,700	40	9,440	19	4,460	
	" 29						21,400	59	12,600	32	6,850	9	1,950	
	" 30						20,200							
	Oct. 1						10,800							
		11.00 a.m.		0.005										

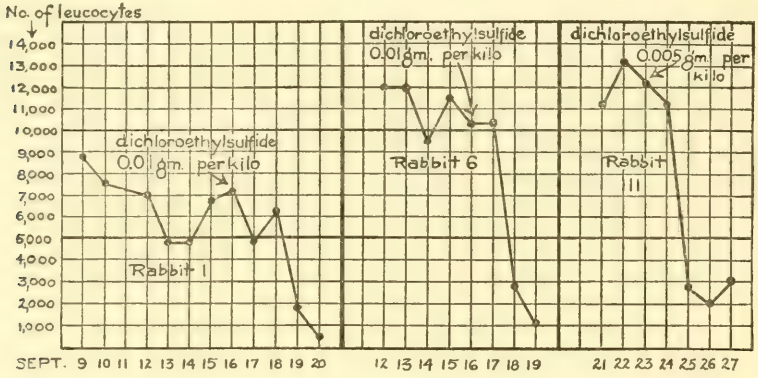
TABLE I—*Concluded.*

Animal No.	Date.	Temperature.	Dose per kilo.	Red blood cells.	Hemoglobin.	Total No. of leucocytes.	Polymorphonuclears.		Lymphocytes.		Large mononuclears.		Other forms.
							Per cent.	No. per c.mm.	Per cent.	No. per c.mm.	Per cent.	No. per c.mm.	
	1918	°C.	*gm.*		*per cent*								
12	Oct. 4					1,000	1	10	82	820	17	170	
	" 5					1,400	2	28	68	967	3	42	Myelocytes 27 per cent.
	" 7					2,100	50	1,050	28	586	6	216	Myelocytes 16 per cent.
							Killed.						
8	Sept. 28					9,800	20	1,960	76	7,450	4	390	
	" 29					10,200	48	4,900	44	4,490	8	810	
	" 30					8,000	22	1,760	64	5,120	14	1,120	
	Oct. 1, 9.00 a.m.					5,000							
	11.00 "		0.005										
	3.30 p.m.					11,000							
	Oct. 2					11,800							
	" 3					9,600	52	5,000	40	3,840	8	768	
	" 4					10,000	70	7,000	25	2,500	5	500	
	" 5					14,500	56	8,100	40	5,800	4	580	
	" 6					13,800	61	8,420	30	4,140	9	1,240	
	" 7					13,200	64	8,450	34	4,490	2	260	
	2.00 p.m.		0.005										
	Oct. 8					9,000	94	8,460	5	450	1	90	
	" 9					900							Large mononuclears too few to count.
	" 10					800							
	" 11						Died.						

13	Sept. 28						13,200	54	7,140	34	4,480	12	1,580	
	" 29						16,600	28	4,650	24	3,990	48	7,800	
	" 30						12,200	24	2,930	51	6,220	25	3,050	
	Oct. 1,	9.00 a.m.					37,000							
		11.00 "		0.005										
		3.15 p.m.					23,000							
	Oct. 2						19,400							
	" 3						5,000	53	2,650	35	1,750	12	600	
	" 4						2,700	14	380	61	1,640	25	670	
	" 5						5,600	18	1,010	41	2,300	20	1,120	Myelocytes 21 per cent. Some nucleated red cells.
	" 6						32,400	23	7,550	33	10,720	38	12,480	Myelocytes 6 per cent. Some nucleated red cells.
	" 7						25,400	85	21,300	7	1,780	6	1,520	Myelocytes 2 per cent. Some nucleated red cells.
		2.00 p.m.		0.005										
	Oct. 8						4,400	76	3,350	23	1,010	1	40	
	" 9						1,800	Cells too few to count.						Large mononuclears.
	" 10						1,800							
	" 11						300							
		3.00 p.m.						Killed.						

degenerated. There are many mitoses. The marrow on the whole is less cellular than normal marrow, and new fat cells are in process of formation. There is much hemosiderin pigment, chiefly intracellular, which may be taken as evidence of previous blood destruction.

After the first injection in Rabbit 13 there ensued a fall in the leucocytes from 37,000 to 2,700 on the 4th day. This was apparently followed by an active regeneration. The count rose again to 32,400, followed by a slight drop to 25,400. At this point a second injection was given, which again was followed by a marked leucopenia, the leucocytes falling to 300 per c.mm., at which point the rabbit was

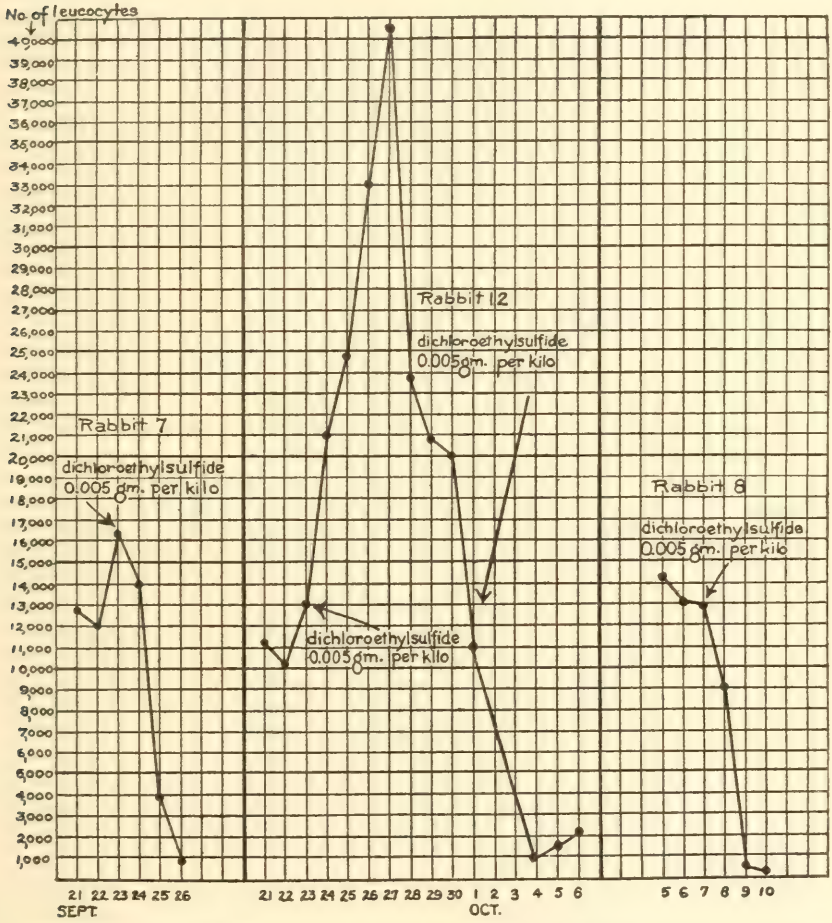


TEXT-FIG. 1. Variations in the total leucocyte count after the intravenous injection of dichloroethylsulfide.[11]

killed. The marrow histologically shows evidence of an initial injury, followed by repair and a fresh destruction of the regenerating cells. There are large areas of almost complete aplasia, similar to that described in Rabbit 8. But there are also hyperplastic areas, composed of aggregates of large cells with poorly staining nuclei, which, under the high power, are found to be distorted and obviously degenerating. It would seem that these hyperplastic foci have been again injured by the second injection of the toxic substance.

[11] The text-figures include only the animals in which blood counts were made during a preliminary period. The fluctuations produced by abrupt change in the surrounding temperature are omitted.

Spleen.—Changes in the follicles—fragmentation of lymphoid cells with phagocytosis of chromatin particles—were seen only in Rabbits 4 and 5. Both these animals died within less than 24 hours after

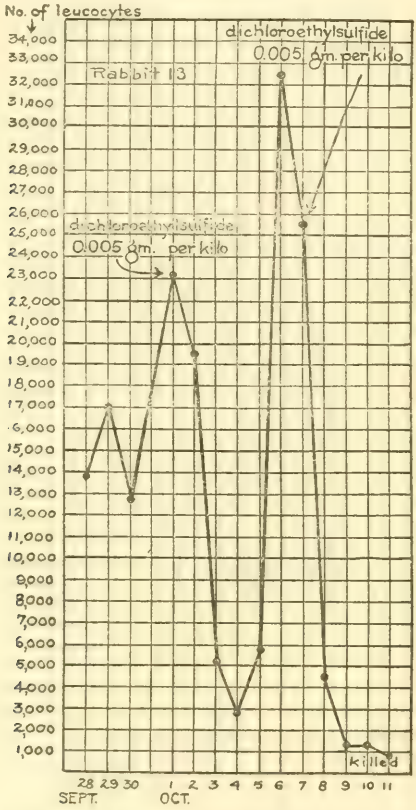


TEXT-FIG. 2. Variations in the total leucocyte count after the intravenous injection of dichloroethylsulfide.

injection. In the remaining rabbits the follicles were normal, or at least showed no signs of either active destruction or excessive proliferation. The sinuses in the majority of the rabbits contained large mononuclear cells laden with blood pigment. The most striking

change, however, was the paucity of free cells in the meshes of the reticulum. The sinuses were separated by strands of cells with pale oval nuclei, evidently belonging to the reticular elements.

Lymphoid Tissue.—The two rabbits (Nos. 4 and 5) which showed acute destruction of the lymphoid cells of the splenic follicles also



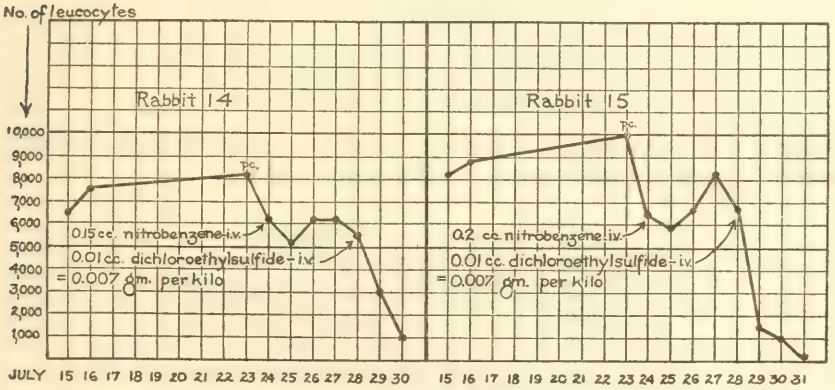
TEXT-FIG. 3. Variations in the total leucocyte count after the intravenous injection of dichloroethylsulfide.

showed cytolysis of the lymphocytes of the thymus and of the intestinal lymphoid tissue.

Because of the great susceptibility of the small thymus cells and of the tissue lymphocytes generally to injurious agents, we are not inclined to regard this as a specific effect of the dichloroethylsulfide,

particularly as it was not present when the destruction of the bone marrow elements was extreme.

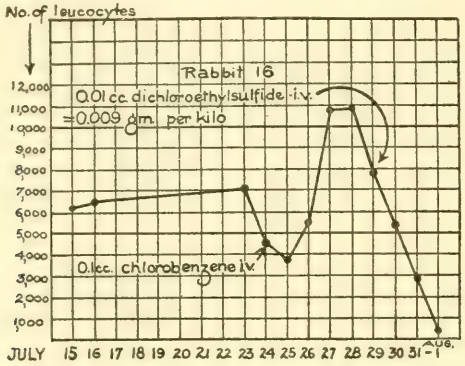
The injury to the blood-forming tissues described bears a close resemblance to the well known action of benzene, with the possible difference that the erythroblastic tissues appear to be less seriously injured than they are with the latter substance. It had been ascertained that the Germans were using nitrobenzene and chlorobenzene as solvents for dichloroethylsulfide in shell fillings, and, indeed, the distillate used by us in our experiments was said to have contained a proportion estimated at roughly from 10 to 20 per cent of these benzene derivatives. Although we were not able to find state-



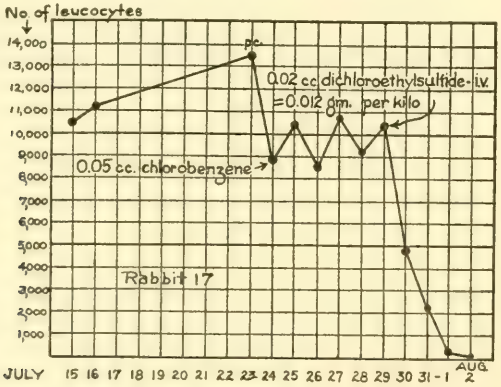
TEXT-FIG. 4. Variations in the total leucocyte count after the intravenous injection of nitrobenzene and dichloroethylsulfide.

ments in the literature as to the leucotoxic action of chlorobenzene and nitrobenzene, doubt naturally arose as to whether the effects which we observed might not have been attributable to the admixture of these solvents, rather than to the dichloroethylsulfide itself, although the minute amounts contained in the doses injected made this rather improbable. The armistice prevented further control experiments at that time. Recently, however, one of us has been able to perform additional experiments on a small series of rabbits, which make it clear that the toxic action upon the leucocytes and bone marrow is due to the dichloroethylsulfide, and not to a possible admixture of chlorobenzene and nitrobenzene.

After a preliminary period of observation two rabbits were injected intravenously with 0.15 and 0.2 cc. of nitrobenzene respectively—doses approximately fifteen and twenty times as large as the total dose of dichloroethylsulfide used in the original experiments. In



TEXT-FIG. 5. Variations in the total leucocyte count following the intravenous injection of chlorobenzene and dichloroethylsulfide.



TEXT-FIG. 6. Variations in the total leucocyte count following the intravenous injection of chlorobenzene and dichloroethylsulfide.

neither of the rabbits did a leucopenia develop. After 4 days, during which time the animals gained in weight and showed no symptoms, they received an intravenous injection of dichloroethylsulfide.[12] In

[12] The sample used was obtained by Dr. Winternitz from the American University, and was stated to be free from nitrobenzene and chlorobenzene.

TABLE II.

Animal No.	Date.	Substance injected.	No. of leucocytes.	Weight.
	1919			*gm.*
16	July 15, 1.00 p.m.		6,175	
	" 16, 2.10 "		6,600	
	" 23, 1.25 "		7,500	
	" 24, 11.10 a.m.		4,750	1,600
	11.15 "	0.1 cc. of chlorobenzene intravenously.		
	" 25, 10.30 "		3,680	1,650
	" 26, 10.00 "		5,500	1,530
	" 27, 10.00 "		10,250	1,670
	" 28, 10.15 "		10,400	1,540
	" 29, 10.00 "		7,900	1,500
	10.50 "	0.01 cc. of dichloroethylsulfide intravenously (0.009 gm. per kilo).		
	" 30, 10.30 "		4,800	1,470
	" 31		2,850	1,450
	Aug. 1		450	1,430
	" 2			Found dead.
14	July 15, 11.30 a.m.		6,500	
	" 16, 1.30 p.m.		7,400	
	" 23, 1.15 "		8,100	
	" 24, 9.50 a.m.		6,250	
	10.05 "	0.15 cc. of nitrobenzene intravenously (1.5 cc. of 10 per cent solution in absolute alcohol plus 3 cc. of distilled water).		
	" 25, 10.00 "		5,300	1,630
	" 26, 9.30 "		6,200	1,730
	" 27, 9.30 "		6,300	1,790
	" 28, 9.30 "		5,650	1,620
	2.35 p. m.	0.01 cc. of dichloroethylsulfide intravenously (0.007 gm. per kilo).		
	" 29, 9.30 a.m.		3,500	1,620
	" 30, 9.30 "		800	1,550
	2.30 p.m.			Killed.

TABLE II—*Concluded.*

Anima No.	Date.	Substance injected.	No. of leucocytes.	Weight.
	1919			*gm.*
15	July 15, 12.30 p.m.		8,300	
	" 16, 1.45 "		8,850	
	" 23, 1.00 "		10,300	
	" 24, 10.30 a.m.		6,400	1,720
	10.35 "	0.2 cc. of nitrobenzene intravenously.		
	" 25, 10.15 "		5,800	1,770
	" 26, 9.45 "		6,700	1,900
	" 27, 9.45 "		7,800	1,860
	" 28, 9.55 "	0.01 cc. of dichloroethylsulfide intravenously (0.007 gm. per kilo).	6,100	1,770
	" 29, 9.45 "		1,500	1,720
	" 30, 9.45 "		1,050	1,720
	" 31, 11.00 "		>200 (counted from heart's blood).	? Killed.
17	" 15, 12.45 p.m.		10,450	
	" 16, 2.45 "		11,300	
	" 23		13,900	
	" 24, 11.30 a.m.		8,900	2,000
	11.35 "	0.05 cc. of chlorobenzene intravenously.		
	" 25, 10.45 "		10,400	2,120
	" 26, 10.10 "		8,000	2,270
	" 27, 10.20 "		10,700	2,430
	" 28, 10.30 "		8,700	2,140
	" 29, 10.30 "		10,400	2,040
	11.00 "	0.02 cc. of dichloroethylsulfide intravenously (0.012 gm. per kilo).		
	" 30, 10.45 "		4,900	1,980
	" 31, 10.00 "		2,400	1,940
	Aug. 1, 10.15 "		300	1,900
	" 2, 10.00 "		>200	1,760 Killed.

each rabbit the injection was followed by the typical leucopenia (Text-fig. 4, Table II).

Two rabbits also were injected with chlorobenzene, the doses used being 0.1 and 0.05 cc. respectively. One of these rabbits before injection showed a slight leucopenia (4,750 per c.mm.), and on the following day the leucocytes fell to 3,680; they then rose to slightly above normal and remained so. The other rabbit showed no significant fluctuation. 5 days after the first injection each rabbit received an intravenous injection of dichloroethylsulfide, the dose being 0.009 and 0.012 gm. respectively, and this was followed by the typical reaction. The leucocyte counts are shown in Text-figs. 5 and 6 and Table II.

These experiments, though not numerous, are clear-cut, and seem to rule out definitely the possible part played by the benzene derivatives in producing these striking effects upon the blood-forming organs.

CONCLUSIONS.

1. The lethal dose of dichloroethylsulfide (distilled from a German yellow cross shell), when injected intravenously into rabbits, is from 0.005 to 0.01 gm. per kilo.

2. Rabbits dying within 24 hours showed extensive hemorrhages and edema of the lungs.

3. Severe lesions of the intestinal tract were present in about one-third of the rabbits.

4. Dichloroethylsulfide injected intravenously is specifically poisonous for the hematopoietic tissues. Severe lesions are caused in the bone marrow, and the number of circulating leucocytes is markedly diminished. In animals surviving the injection regeneration occurs. The granular cells of the bone marrow seem to be more sensitive than the lymphoid cells and the erythrocytes.

5. The effect upon the blood and hematopoietic tissues is not due to the admixture of nitrobenzene or chlorobenzene in the shell filling. Injection of these substances in animals in amounts many times greater than the total dose of dichloroethylsulfide used produced no changes in the blood picture, and the subsequent injection of dichloroethylsulfide free from these solvents produced a typical reaction.

EXPLANATION OF PLATE 10.

FIG. 1. Rabbit 8. Bone marrow of femur 4 days after the second injection of 0.005 gm. of dichloroethylsulfide. The sinuses (*bld. ves.*) are widely dilated and filled with erythrocytes. They contain practically no nucleated cells. The endothelial cells (*end.*) are preserved. There are scattered megacaryocytes (*mg.*) with darkly stained nuclei. There are also degenerating cells with fragmented chromatin (*pyc.*). Myeloblasts, myelocytes, and adult granulocytes of all types have disappeared, and there are only occasional small mononuclear elements. The space between the sinuses is occupied by adult and immature fat cells (*f.*).

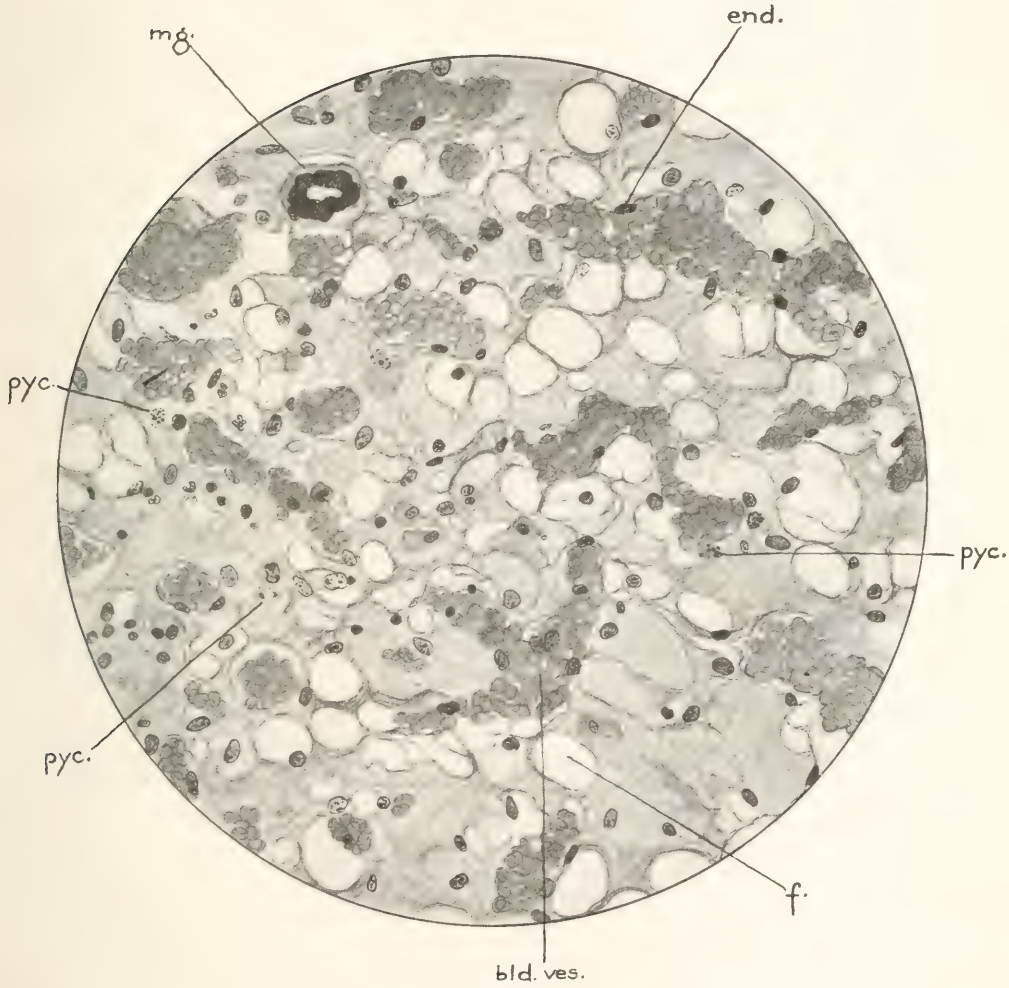


FIG. 1.

(Pappenheimer and Vance: Intravenous injections of dichloroethylsulfide.)

Reprinted from the Proceedings of the Society for Experimental Biology and Medicine, 1919, xvi, pp. 92–93.

53 (1428)

The effects of intravenous injections of dichlorethylsulphide in rabbits.

By Alwin M. Pappenheimer.

[*From Columbia University, New York City.*]

The effect of intravenous injections of dichlorethylsulphide (mustard gas) was studied in a small series of rabbits. The minimum lethal dose was found to be from 0.005 gm. to 0.01 gm. per kilo. The injection was followed by emaciation, diarrhea, and, in animals dying within a few hours following the injection, extreme restlessness, incoördinate movements, retraction of the head, and transient spasticity, but no definite paralyses or convulsions. Animals dying within twenty-four hours or so showed irregular pulmonary edema. The most interesting effects were found in the hemato-poietic system. Usually on the second day after the injection, the circulating blood showed a marked leukopenia, which in the terminal stages became extreme, leukocytes falling to 1,000 per cubic mm. or less. In animals which recovered there followed a gradual restoration to the original level. The leukopenia was accompanied by a relative but not absolute, mononucleosis. The erythrocytes appeared to be less severely injured.

A study of the bone marrow in these animals shows an effect comparable to that of benzol. There is early destruction of the cells of the granulocyte series and in some animals an extraordinary depletion of the bone marrow. Animals which partially recovered from the initial injection and were then killed, showed active regenerative changes in the bone marrow.

There is a possibility that these effects may have been due to the chlor-benzine or nitro-benzine used as a solvent in German gas shells, and carried over in the distillate used for injection. In view of the small amount of such impurity present, this does not seem likely, but there was no opportunity to repeat the experiments with dichlorethylsulphide completely freed from this solvent.

The experiments were carried out in collaboration with Capt. Morgan B. Vance, M.C., at Hanlon Field, Chaumont, France.

FURTHER STUDIES ON THE IMPORTANCE OF THE LYMPHOCYTE IN CANCER IMMUNITY.*

M. J. SITTENFIELD.

(*From the Department of Pathology, College of Physicians and Surgeons, Columbia University.*)

In a previous communication[1] were reported experiments which failed to induce immunity in rats inoculated with Flexner Jobling rat carcinoma, when artificial hyperlymphocytosis was produced on the one hand; or to alter their resistance to F. J. R. C. by depletion of the lymphoid cells in the circulating blood on the other. To review them briefly: In one set of white rats a high lymphoid count in the blood was induced by repeated injections of pilocarpin, causing a general rise of the small mononuclear lymphocyte from twenty-five or thirty per cent to fifty-five and even sixty per cent. At this period they were inoculated with F. J. R. C. and the resultant percentage of takes was the same in the controls as in the experimental rats. In the second set of experiments a leucocytic cream from rats, which had previously been treated with small or stimulating doses of x-ray, was injected intravenously into normal rats so that a high lymphocyte count in the general circulation resulted, and when these were inoculated with the F. J. R. C. they maintained their normal susceptibility, as the percentage of successes did not differ from that in the controls, *i.e.*, the artificial stimulation of the small mononuclear lymphocyte in both sets of experiments exhibited no manifestations of immunity. In a third set of experiments a number of nullers, whose absolute immunity was tested by several inoculations with the F. J. R. C., were rayed repeatedly, almost depleting them of their lymphoid elements, by causing the lymphocyte in the blood to fall to four per cent. When these were subsequently inoculated with the F. J. R. C. their state of immunity was not in the least altered. In other words, the susceptibility to F. J. R. C. in the one set of rats remained the same

* Received for publication June 1, 1918.

in spite of the hyperlymphocytosis induced, and in the other the state of resistance to F. J. R. C. was maintained in the face of almost complete depletion of the lymphoid elements in the rats. These results were so contrary to those reported by Murphy and Morton[2] that further experiments were advisable in order to clear up our differences, inasmuch as an important factor in immunity to cancer was concerned. Therefore, several other experiments were conducted, following closely the work of Murphy, except that F. J. R. C. was used in this work. In eighty-nine rats inoculated with F. J. R. C. the tumor was excised completely, ranging at different periods from ten to twenty-eight days. While the tumor was out a small or stimulating dose of x-ray was administered. They were re-inoculated with their own tumor in the opposite axilla. Careful blood counts were made before and after the raying to determine the degree of lymphocytosis, and in all but two the tumor grew at the site of the second inoculation. In none was there a recurrence at the original site of the tumor.

CHART I.

	Number of Rats.	Small Mononuclears before Raying.	Small Mononuclears after Raying.	Percentage of Takes.	Percentage of Recurrences at Original Site.
		Per Cent.	*Per Cent.*		
Experiment . .	89	11–35	26–70	98	0
Controls	49	10–54		97.9	

In forty-seven rats incomplete excision of the tumor was purposely performed, and the same procedure followed as above, namely, the same x-ray dose was administered, while the tumor was out, and also re-inoculated with the same tumor in the opposite axilla, and in these the tumor grew in every one at the site of the second inoculation and recurred in forty-eight per cent at the site of excision. In brief, the results corroborated the findings reported by me at the last meeting of this society.

CHART II.

	Number of Rats.	Small Mononuclears before Raying.	Small Mononuclears after Raying.	Percentage of Takes.	Percentage of Recurrences at Original Site.
		Per Cent.	*Per Cent.*		
Experiment . .	47	10–37	24–65	89.3	48
Controls	29	11–35		90	

These results seemed conclusive that the degree of lymphocytosis had very little to do with immunity to Flexner Jobling rat carcinoma, and to test it further, it seemed advisable to repeat Murphy's experiment in mice. I wish here to express my thanks for the courtesy extended to me by Dr. Murphy, supplying me with his mouse tumor, and it will be of interest to note that the results obtained in mice were quite similar to those reported by Murphy and Morton,[3] excepting perhaps that our tumors recurred at the usual periods, and the latency of four to five weeks after the raying could not be confirmed. Careful blood counts were made in these mice before and after raying, the increase of the small mononuclear lymphocyte ranged in average from thirty to nearly fifty and fifty-five per cent in a total of 14,000 to 18,000 whites. The red blood cells also were quite high, ranging from five to seven millions. This high lymphoid count generally reached its optimum in from forty-eight to seventy-two hours and continued for about five to six days. The tumor generally was of moderate size on the tenth to twelfth day. Out of ninety-three mice treated with a small dose of x-ray, while the tumor was out of the body, and then re-inoculated with their own tumor, fifty-four tumors recurred, eighteen died during the experiment and the rest, or twenty-one, remained free.

CHART III.

	Number of Mice.	Small Mononuclears before Raying.	Small Mononuclears after Raying.	Percentage of Takes.	Number Dying during Experiment.
		Per Cent.	*Per Cent.*		
Experiment . .	93	5–27	28–56	58	18
Controls . . .	50	5–16			60

Woglom reported at the last meeting of the American Association for Cancer Research that the histological picture of lymph nodes of immune rats did not differ from that of normal susceptible ones. In my own clinical observations I have encountered many cases of advanced carcinoma with metastasis where a very high lymphoid count continued throughout the course of the disease. Furthermore, in malignant lymphomata the carcinoma has a predilection for the lymph glands throughout the body. The same is true of lympho-sarcomata. Therefore it is difficult to conceive how the immunizing factor can be attributed to the small mononuclear lymphocyte.

It must appear to every one that the behavior of tumor growth in white rats inoculated with F. J. R. C. was not influenced in the slightest by the high lymphoid count.

The agent which causes immunity to some degree in mice and fails entirely in rats must necessarily be of negligible value.

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ATYPICAL HORSESHOE KIDNEY

J. G. GARLOCK

(*From the Pathological Laboratory of the Presbyterian Hospital, New York*)

Anomalies of the kidney fall into several well-recognized groups, of which the more important are unilateral congenital atrophy, fused kidney, solitary kidney, and lobulated kidney. While this specimen does not correspond exactly with any of the commoner types described, it may be regarded as an atypical horseshoe kidney.

In the clinical history of the case there was nothing to indi-

cate the existence of renal anomaly. The patient, a negro man of 52 years, had been admitted to the hospital four times during the last two years of his life, complaining generally of dyspnea, edema of extremities, and lassitude. Physical examination

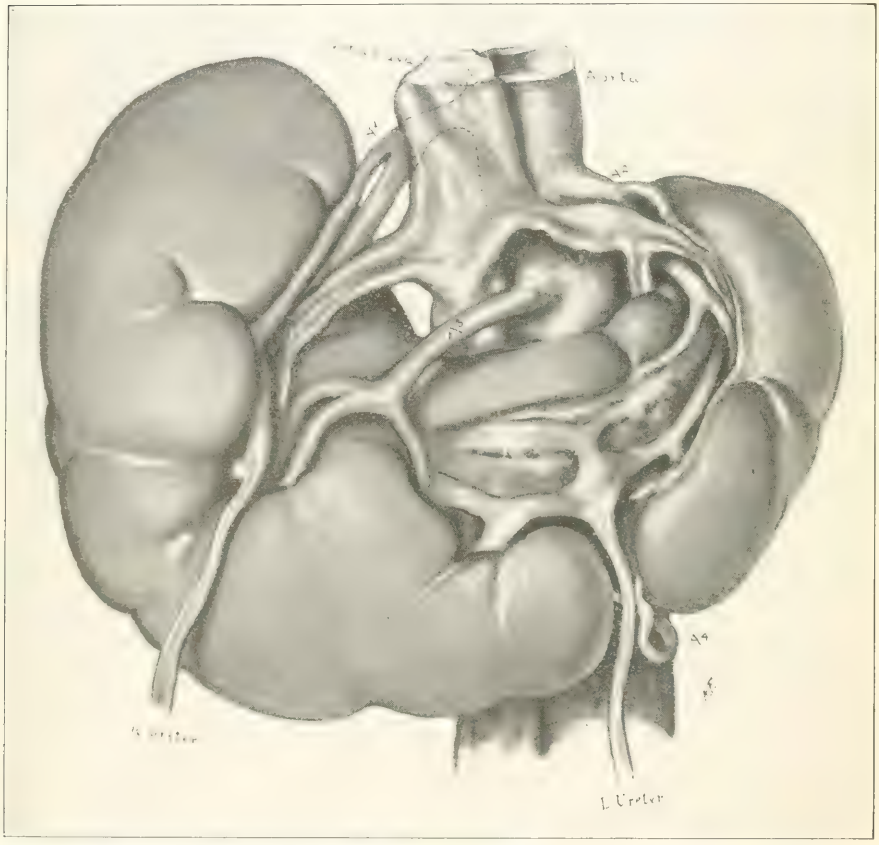

FIG. 1. Atypical horseshoe kidney. A^1, A^2, A^3, A^4, renal arteries.

showed evidences of arteriosclerosis and chronic myocarditis with cardiac insufficiency and glycosuria. The circulatory condition generally improved under treatment, and the sugar disappeared from the urine with a proper diet. During his last stay in the hospital the sugar persisted in the urine, and gangrene of

the right foot developed, followed by general anasarca, coma, and death. The specific gravity of the urine was 1.030 to 1.040, and there was a trace of albumin, but no casts or cells. The systolic blood pressure was 166, the diastolic 70.

At autopsy there was found, in addition to the renal anomaly, a generalized arteriosclerosis, involving especially the aorta, and the coronary and cerebral vessels, chronic passive congestion of the organs, and a chronic intralobular pancreatitis. Only the kidneys, however, need be described. When the intestines were removed, a big mass of kidney tissue was seen to the right of the vertebral column, with only a small mass in the region of the left kidney. Kidneys, ureters, and bladder were then removed *en masse* and carefully dissected. The right kidney was found to be fully twice as large as the left, and there was a broad mass of kidney substance extending between the two from the hilus to the lower poles. The upper part of this uniting band projected further forward than the lower, as is shown in the accompanying illustration (Fig. 1). Posteriorly, there was a uniform sheet of kidney tissue connecting the two organs. The renal vessels and ureters showed also certain abnormalities in their origin and branching. There were four arteries. The first (A^1) left the aorta rather high up on the right side and divided immediately into two branches, which supplied the upper portion of the right kidney. The second (A^2) came off on the left side about opposite the left renal vein and gave branches to the small left kidney and to a part of the medial sheet of kidney tissue. The third (A^3) was an anterior branch of the aorta, starting just below the renal veins and supplying the lower portion of the right kidney. The fourth (A^4) was given off at a point about opposite the lower border of the kidney, that is, only 4 cm. above the bifurcation. It supplied the lower portion of the renal isthmus. The veins showed no definite abnormalities.

The two ureters pursued a normal course after leaving the kidney, but were anomalous in their beginnings. The right drained only the upper portion of the right kidney, while the left was formed by branches from the left kidney, the lower portion

of the right, and the medial communicating mass of kidney tissue.

It is evident from this brief description that this specimen is not a typical horseshoe kidney, although it undoubtedly belongs to that general group. Eisendrath divides fused kidneys into three classes: (1) Typical horseshoe kidney with fusion confined to the lower poles; (2) intermediate form of solitary kidney; (3) disc-like kidney, where both organs are fused into one disc-shaped mass. This specimen falls into the second class of intermediate solitary kidney. A similar case has been described by Küster.

Horseshoe kidneys are not rare. They constitute by far the most common form of renal malformation. Keyes, in 11,000 autopsies, found 53 malformed kidneys, and 16 of these were of the horseshoe type. The origin of the fused kidney is fairly well understood. The two mesothelial bodies, which are the "anlagen" of the renal parenchyma, unite with the outgrowths from the Wolffian ducts, which form the collecting radicals of the renal system. These primitive structures gradually ascend from the pelvis to their final resting place. It is before this ascent or during it that the lower poles may approximate and become fused. The amount of kidney tissue involved in this disturbance determines the character and conformation of the anomaly. According to Charles Mayo, anomalies of the blood vessels are due to the persistence of some of the vessels of the plexus which surrounds the renal "anlage" during embryonic life.

The clinical significance of fused kidneys has received a good deal of attention. Rovsing, after studying four cases, constructed a clinical picture of horseshoe kidney, the chief feature of which is pain across the abdomen induced by exercise, exaggerated when the patient bends forward, and relieved by lying down. He believes that such masses of kidney tissue can be palpated in front of the great vessels in individuals with lax abdominal walls. Eisendrath has called attention to the relative frequency of hydronephrosis and pyonephrosis in horseshoe kid-

neys, and cites two cases in which a diagnosis of horseshoe kidney was made before operation.

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Discussion:

DR. A. L. LOURIA: The clinical picture that Dr. Garlock suggests interests me because of a case I saw two years ago. The patient was a girl, twenty years old, who complained of pain just below the umbilicus. She was well nourished and did not look ill. Physical examination revealed nothing beyond a mass just above the level of the sacrum, about two inches broad, tender, and definitely overlying the aorta and pressing upon it, as evidenced by a bruit and a transmitted, non-expansile pulsation. The diagnosis of horseshoe kidney was made, and this was subsequently confirmed at operation at the Jewish Hospital.

DR. MOSCHCOWITZ: It seems to me it would be very interesting to determine the relation of the pelves by *x*-raying the kidney after the injection of collargol.

DR. LEON LOURIA: In the case just mentioned, which was sent to the Jewish Hospital of Brooklyn, the diagnosis was made before operation. We injected thorium, which affirmed the diagnosis and gave a beautiful picture of the relation of the pelves of the kidneys. The patient was operated upon successfully and relieved of her pain. It is interesting to note further that, while her symptoms were temporarily alleviated, she returned to the hospital some time later with a recurrence of the abdominal pain. On examination a mass, similar to that before operation, was readily palpated. It appears that the severed poles had reunited. Unfortunately her symptoms subsided during her stay in the hospital, and she refused a second operation, so that we were unable to substantiate our assumption. It would be interesting to learn if surgery can completely relieve such anomalies.

DR. WILLIAM LINDER: My only experience in operating upon a horseshoe kidney is in the case referred to me by Dr. Leon Louria. A previous diagnosis having been made, I made a right rectus incision and entered the peritoneum. After the intestines had been thoroughly packed away, the posterior layer of the peritoneum overlying the kidney was incised and the kidney exposed. The blood supply was studied and the ureters identified; the kidney was then severed and a wedge-shape piece removed from each part in order to reconstruct the lower pole. I was not satisfied until we

could place the left kidney to the left of the spinal column. Subsequent examination of the patient revealed a probable reunion of the severed kidney. I believe that the fibrous capsule which enveloped the entire kidney had probably pulled the poles together. In the next case, I propose making a median incision and going through intraperitoneally in order that I may be able to fix one kidney higher and decapsulate the other, so that a union can not take place. Perhaps we can thus avoid the fusion of the two poles.

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A POLYPOID MYXOMA OF THE HEART

HENRY W. LOURIA

(*From the Pathological Laboratory of the Presbyterian Hospital, New York*)

The specimen which I wish to present is an example of the so-called polypoid myxoma of the heart, a very rare tumor,

though probably the most common one originating in the heart, which, as you know, is rarely the seat of primary new growths. In a series of twenty-eight cardiac neoplasms collected by Berthenson in 1895, myxoma ranked second in frequency; but in a larger series of ninety-one cases, reviewed by Lind in 1909, this tumor stood first, there being eighteen cases of myxoma and thirteen of sarcoma, the second in frequency. Of the total number of myxomata of the heart reported up to the present time, only three have been described in American literature, whereas at least twenty have been recorded in the German journals of the past twenty-five years. This tumor appears to have excited special interest among the German pathologists, not so much because of its comparative rarity among tumors in general, as because of the discussion which arose over the question as to whether or not the tumor is a true neoplasm. I shall take up this question after presenting my case.

The patient from whom the specimen was obtained was a woman, aged 53, who was admitted to the Presbyterian Hospital in July, 1917, complaining of shortness of breath and general weakness of four months' duration. Her family history was irrelevant, her past history negative. She had never had an attack of acute rheumatic fever, and there was no evidence in the history of previous circulatory disturbance. Her chief symptoms, weakness and shortness of breath, began soon after a severe cold contracted during March, 1917, four months before death. Three days before admission to the hospital, weakness increased and dyspnea became very distressing. The findings on physical examination upon entrance were those referable to a state of cardiac insufficiency. The heart was definitely enlarged; the area of cardiac dullness extended 13 cm. to the left of the midline in the fifth intercostal space. Heart sounds were loud and ringing; there were no murmurs. On the third day in the hospital, after turning suddenly in bed, the patient stopped breathing; the heart continued beating feebly for a few moments, and then ceased.

The autopsy was performed six hours after death. The

findings, except for those in the heart, were unimportant. There was moderate chronic passive congestion of all the viscera.

FIG. 1. Polypoid myxoma of heart. Photograph of gross specimen. Note size and location of tumor, nodular surface, and deep anterior furrow.

The right side of the heart was found in a state of marked dilatation, while the left ventricle was firmly contracted. On opening the left auricle, its wall was seen to be considerably hypertrophied, measuring 3 mm. or more in thickness. Occupy-

ing practically its entire cavity was a large, glistening, gelatinous tumor mass measuring 5 cm. in width and 6.5 cm. in length (Fig. 1). It was attached by a narrow stalk, 1 cm. in diameter, to the interauricular septum just anterior to the fossa ovalis. The most striking features of the tumor, which immediately impressed those present at the autopsy, were its remarkable translucency and the glossy character of its surface, different from the dull, lusterless appearance of the ordinary cardiac thrombus. Covering the entire surface of the tumor were numerous, slender, villous-like projections, characteristic of papillomatous tumors. Along one of the lateral surfaces there was a narrow, but fairly deep furrow, which, judging from its general direction, might have served during life to conduct the blood from the upper and back part of the left atrium, downwards and forwards to the mitral orifice.

The histological picture of the tumor was quite interesting. The free margin of the section was very irregular, corresponding to the villous-like projections on the gross specimen. The surface was covered by a single layer of cells with deep blue-staining cytoplasm, and oval vesicular nuclei (Fig. 2). The cells were more flattened at the summit of the projections than in the depths of the intervening depressions. Many of the cells possessed branching protoplasmic processes which extended into the substance of the tumor. The general appearance of the cells suggested that they were not endothelial cells of the ordinary type, but connective tissue cells in the process of adaptation to pressure conditions within the atrium. The histological structure of the tumor proper varied in different areas. Immediately beneath the surface, there was seen a loose blue-staining matrix in which were scattered many branching connective-tissue cells. The picture was practically identical with that of Wharton's jelly of the umbilical cord. Towards the deeper parts of the tumor, the matrix stained less deeply than in the superficial areas, and in addition there appeared large, irregular spaces lined by cells similar to those covering the surface. Some of these spaces contained well-preserved red blood cells. Here and there, also,

were small discrete masses of pink-staining fibrin, which showed no tendency to organization and had the appearance of being freshly formed. They did not seem to bear any recognizable relation to the other structural elements of the tumor. At the stalk there was a considerable amount of yellow granular pigment (hemosiderin) not found in other parts of the tumor; most of it had been taken up by large phagocytes.

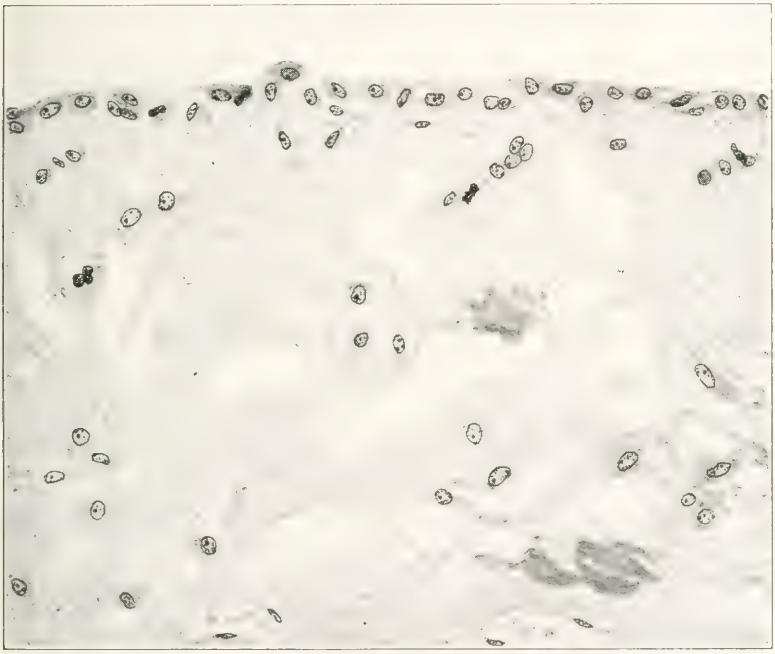

FIG. 2. Polypoid myxoma of heart. Drawing showing layer of endothelial cells covering surface of tumor, and loose, relatively non-cellular myxomatous tissue beneath.

The question now arises as to the interpretation of this pathological specimen. In practically all its characteristics it is identical with the majority of myxomata of the heart reported in the German literature. It is needless to point out that the gross appearance of the tumor is entirely different from that of an ordinary cardiac thrombus. Histologically, the presence of

isolated fibrin masses and blood pigment would seem to lend support to the view that the "tumor" is nothing more than an organizing thrombus, but in view of the absence of the other histological characteristics of a thrombus, one is justified, I think, in accepting a different explanation of the origin of the fibrin and pigment.

In the German literature of the past twenty-five years there has been considerable discussion as to the nature of the cardiac polyp; and it may not be amiss at this point to review briefly the main ideas which have been advanced. In his several reviews of the subject, Thorel has maintained that the cardiac polyp, such as I have described, is not a true tumor, but has its origin in a cardiac thrombus. He believes that the connective tissue of the organized thrombus undergoes edematous changes with transudation of fluid into the space between the cells and fibers, and that subsequently chemical changes take place in the edematous fluid, resulting in the production of mucin. This process is termed by Thorel "myxomatous metamorphosis" or "myxomatous degeneration." This change results in a tissue differing greatly in appearance, macroscopically and microscopically, from the ordinary organizing thrombus. Among those opposed to this view is Ribbert, who maintains that the cardiac polyp is a true tumor. He is inclined to doubt the occurrence of "myxomatous metamorphosis"; and believes, on the contrary, that myxomatous tissue is an independent variety of connective tissue, and that tumors composed either wholly or in part of myxomatous tissue should not be looked upon as the result of secondary change in some other tissue but as the product of true tumor growth. It is obvious that the whole question of the nature of cardiac polyp hinges upon the existence or nonexistence of myxomatous degeneration.

Quite a number of other able pathologists, including MacCallum, regard the occurrence of true myxomatous degeneration as very doubtful. It is their opinion that the burden of proof rests upon those who maintain that the cardiac polyp is simply an organized thrombus with myxomatous degeneration, and that

until such proof is submitted the cardiac polyp should be classified as a true myxoma.

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Discussion:

Dr. Lambert: I was present at the autopsy which Mr. Louria has described and was very much interested in the findings. There is one point upon which I do not think he has laid sufficient emphasis. That is the absence of any cause of circulatory disturbance other than the polypoid tumor of the heart. If, as some writers have maintained, these tumors are nothing more than organized thrombi, one should find in every case a cause for thrombus formation. A second point which might be emphasized is that these polypoid tumors are usually attached to the inner wall of the auricle just anterior to the fossa ovalis, an area, as Ribbert has pointed out, in which an embryonic displacement of tissues favorable to tumor development is likely to occur.

Dr. Moschcowitz: I should like to ask Mr. Louria if the patient had delirium cordis.

Mr. Louria: I did not see the patient clinically.

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MYOCARDITIS IN CASES OF ENDOCARDITIS

ALFRED G. LANGMANN, M.D.

Out of a total of about 400 autopsies in the Presbyterian Hospital series, the following fifty-four cases, showing definite heart lesions, were selected for study. The listing of these cases in various groups was, in a number of instances, a purely arbitrary matter, and so it happens that the same case will appear in one group because of its chronic lesions and in another group because of its acute lesions. In arriving at this classification the history of past and present illnesses, the gross findings in the heart at autopsy, and the results of bacteriological and clinical tests both during life and after death were taken as a basis.

Accordingly the cases group themselves as follows:

I. Acute rheumatic endocarditis (with or without chronic lesions)	10
II. Acute *Streptococcus viridans* endocarditis (with or without chronic lesions)	5
III. Acute pneumococcic endocarditis (with or without chronic lesions)	5
IV. Acute staphylococcic endocarditis (with or without chronic lesions)	2
V. Chronic rheumatic endocarditis	18
VI. Chronic non-rheumatic endocarditis	11
VII. Syphilitic endo- and myocarditis	6

All these cases were studied with especial reference to the myocardial changes in an endeavor to substantiate the above classification by more or less distinctive findings in the myocardium.

I. *Acute Rheumatic Cases:* In all of the ten cases Aschoff bodies were found in the myocardium. In seven of these, they were numerous, and in one, very numerous. Two of the cases showed only one Aschoff body respectively in several slides; but in both cases the cells composing the nodule and also the grouping were quite typical. In fact, in all but one case (8348) these nodules were found to be quite typical. As a rule, they were found in the midst of muscle tissue, surrounded by a slight or moderate amount of fibrous tissue, or lying adjacent to a blood-

vessel, in certain places the cells appearing strung out alongside the blood-vessel wall. The cells composing these nodules varied somewhat in character, some of them having a fairly deep bluish purple staining cytoplasm with irregular but more or less definite cell outline, others staining a faint purple and showing an extremely hazy and vague cell boundary. The character of the nucleus, also, varied to some extent, some of the nuclei taking on a fairly dark, more or less homogeneous stain, others, somewhat larger in size, appeared as dark circles or ovals inclosing a light field in the center of which the chromatin was gathered in a round or, more often, in an elongated mass.

In practically all the cases there was but slight fibrosis associated with the nodules and in only four cases were definite myocardial scars noted. In two of the cases (8238 and 8457) typical Aschoff bodies were found situated directly in the thickened endocardium, one such nodule causing a distinct bulging of the endocardium. In all of the nodules found, it was noted that the muscle fibers in the immediate vicinity, *i. e.*, those abutting on the tissue in which the nodule was situated, appeared healthy and stained deeply with eosin. There was no gradual transition between these apparently healthy fibers and the Aschoff cells, as one might expect if these latter were derived from degenerating muscle fibers. Furthermore, these cells were in closer approximation to the vessel wall than is the case with normal muscle fibers. In the majority of the cases, the cells resembled most closely the endothelial or mononuclear wandering cells, but the characteristic grouping of the chromatin in the center of the vesicular nucleus was in many instances very striking. In a number of cases, multinucleated cells were seen associated with the mononuclear variety in these nodules. The nuclei of these cells were invariably of the very intense and more or less homogeneous staining variety. In all of the cases the associated round-cell infiltration was very slight.

It should be noted that in three of the cases *Streptococcus viridans* was recovered at autopsy. In one case (8256) the diagnosis of pneumococcus bacteremia was made and pneumococcus

was recovered from the blood during life and from the pericardium post mortem.

Of the ten cases, six gave a history of rheumatic fever at some time during life, and two of these an additional history of chorea. Case 8507 which gave a past history of 6 attacks of rheumatic fever showed relatively few and small myocardial scars and only one Aschoff body was found in several slides. The one case (8348) in which the Aschoff bodies were considered not typical, though suggestive, gave a history of "heart trouble" following scarlet fever, but no rheumatic or choreic history. Only one case showed entire absence of pericarditis; while six had a fairly marked pericardial affection.

(N. B. In those cases in which the Aschoff bodies were quite numerous they occurred not only in the region near the base of the left ventricle but also in other parts of both left and right ventricles and in the interventricular septum.)

II. *Streptococcus Viridans Cases:* These cases show no striking myocardial changes. Several show small focal collections of polynuclear and mononuclear wandering cells; some of these near and others apparently away from blood-vessels. There are, also, small areas of necrotic muscle fibers in which only the sarcolemma and a small pyknotic nucleus remain as evidence of a pre-existing normal muscle fiber. As a rule, there is no cellular infiltration associated with these necrotic areas. In three of the cases small emboli are seen in one vessel of each of the respective cases, and in only one of these cases a fairly marked infiltration of polynuclear cells is seen about the vessel wall.

V. *Chronic Rheumatic Cases:* Of the eighteen cases in this group, all but two gave a more or less definite history of rheumatic fever, and the two exceptions, a history of chorea. Ten of the cases show myocardial scarring of varying degrees, quite pronounced in two instances, and in two cases typical Aschoff bodies were noted. In a third case (8273) numerous small groups of darkly stained mononuclear cells were seen near the endocardium, adjacent to blood-vessels and in the midst of muscle. These cells resemble Aschoff cells but the majority of

them are not characteristic and the grouping is not typical. Pericarditis was present in only eight instances.

In case 8482 in which numerous Aschoff bodies were found there was no evidence of an acute valvular lesion. The patient came to the hospital complaining of abdominal pain and swelling of face, abdomen, and legs of several months' duration, and also of orthopnea and precordial pain. He gave a history of rheumatic arthritis six years before.

The other case (8473) which showed one fairly typical Aschoff body, associated with which are numerous lymphocytes and one eosinophile, gave a history of rheumatic arthritis twenty-nine years before and no subsequent attacks. The myocardium in this case showed several small areas of scarring whereas the preceding case showed none.

III. *Pneumococcus Cases:* Except for one or two collections of lymphocytes the musculature showed nothing especially noteworthy except in one case (8256) which has already been considered in the acute rheumatic group. In this instance, the diagnosis of subacute articular rheumatism was made clinically, although the cause of death was bronchopneumonia and pneumococcus bacteremia. The myocardium in this case showed numerous typical Aschoff bodies. Moreover, this case showed a definite pericarditis, whereas the other four cases showed no such lesion.

IV. *Staphylococcus Cases:* Of the seven cases in this group only three showed definite miliary abscesses scattered throughout the muscle, containing a bluish mass of bacteria at their center. No scars were seen except in one case. The other cases showed no inflammatory changes and no connective tissue increase. (N. B. In each of these cases a staphylococcus bacteremia was found and all but one showed definite lesions of the heart valves.)

VI. *Chronic Non-Rheumatic Cases:* None of the eleven cases gave any history of rheumatic fever or chorea and only four of tonsillitis or sore throat. Three of these four cases showed definite myocardial scars. Another case (8544), also, showed

marked scarring, but in this case there was an associated general arteriosclerosis.

VII. *Syphilitic Cases:* Of the five cases, three showed a cellular infiltration consisting chiefly of lymphocytes and some polynuclear leucocytes. This infiltration was found either about the vessels or in the regions of degenerating muscle fibers which in several sections were quite prominent. One case (8439) showed large mononuclear cells (? wandering cells) scattered here and there between muscle fibers and frequently arranged in cords and even double strands. It is difficult to determine whether these cells were inclosed within capillaries or whether they were simply lining the space between separated muscle fibers. Many of the muscle fibers showed no striations whatsoever, and some of the other cases showed marked changes in their staining reaction. In three instances, marked fibrous scarring was noticed.

CONCLUSIONS

1. All cases of *acute* rheumatic endocarditis studied, ten in number, showed typical Aschoff bodies in the myocardium. Aschoff bodies were found also in two out of eighteen cases of so-called *chronic* rheumatic endocarditis; that is, cases of chronic cardiac valvular disease with a definite history of rheumatic fever or chorea. They could not be demonstrated in any other type of endocarditis.

2. The cases of chronic rheumatic endocarditis did not show myocardial changes which would differentiate them from the non-rheumatic group, with the exception of two in which Aschoff bodies were found. Myocardial scars were present in about fifty per cent. of all cases of chronic endocarditis, and were as common in the non-rheumatic as in the rheumatic group.

3. In three out of seven cases of acute staphylococcus endocarditis, miliary abscesses and bacterial emboli were demonstrated in the myocardium. None of the remaining types of endocarditis studied showed any characteristic myocardial lesion.

Acute Rheumatic Cases

Autopsy No.	Age	Sex	Rheum. Fever	Chorea	Tonsillitis	Pericarditis	Valve Lesions	Scars	Aschoff Bodies	
8238	12	F	−	−	+	++	+	+	++	
8348	21	F	−	−	−	++	+	−	+	
8380	17	F	++	−	−	+++	+	−	++	
8444	9	M	−	−	−?	++	+	−	++	
8450	12	F	+	−	−	+	+	+	+++	
8457	4	F	+	−	−	+sl.	+	−	++	
8507	23	F	++	+	−	−	+	+	+	
8568	18	F	+	+	−	++	+	+	++	
8588	11	F	−	−	+	++	+	−	++	
8256	23	F	+	−	+	+	+	−	++	Also listed in Pneumococcus Group.

Streptococcus viridans Cases

Autopsy No.	Age	Sex	Rheum. Fever	Chorea	Pericarditis	Valve Lesions	Scars	Aschoff Bodies	Emboli	
8275	22	F	−	−	+	+	−	−	+	
8442	38	M	+?	−	++	+	+	−	+	
8518	10	F	−	+	+	+	+	−	+	
8562	30	F	+	−	+	+	−	−	−	Also listed in Chr. Rheum. Group.
8641	24	M	+?	−	−	+	+	−	−	

Pneumococcus Cases

Autopsy No.	Age	Sex	Rheum Fever	Tonsillitis	Pericarditis	Valve Lesions	Scars	Aschoff Bodies	Abscesses	
8343	26	F	+	+	−	+	−	−	−	Also listed in Chr. Rheum. Group.
8256	23	F	+	+	+	+	−	+	−	Also listed in Ac. Rheum. Group.
8425	21	F	−	−	−	+	+	−	−	
8536	55	M	−	−	−	+	−	−	−	
8570	39	F	−	+	+sl.	+	−	−	−	

Staphylococcus Cases

Autopsy No.	Age	Sex	Rheum. Fever	Chorea	Tonsillitis	Pericarditis	Valve Lesions	Scars	Aschoff Bodies	Abscesses	
8494	32	F	−	−	+	−	+	−	−	++	
8502	25	F	−	−	−	−	+	−	−	−	
8295	23	F	−	−	−	+sl.	+	−	−	−	
8270	46	M	−	−	−	−	+	−	−	−	
8386	36	M	−	−	−	−	+sl.	+	−	+	
8387	19	F	+?	−	+	−	+	−	−	+	
8393	20	M	−	−	−	−	−	−	−	−	

Chronic Rheumatic Cases

Autopsy No.	Age	Sex	Rheum. Fever	Chorea	Tonsil-litis	Pericar-ditis	Valve Lesions	Scars	Aschoff Bodies	
8254	24	M	+	−	+?	−	+	+	−	
8255	34	F	+	−	−	++	+	+	−	
8273	39	M	+	−	−	−	+	+	+?	
8300	17	M	−	+	−	++	+	−	−	
8312	61	M	+	−	−	+	+	+	−	
8326	28	F	+	−	−	−	+	−	−	
8332	62	M	+	−	−	+	+	++	−	
8415	23	M	+	−	−	+sl.	+	++	−	
8419	20	F	−	+	−	−	+	−	−	
8441	51	F	+	−	−	−	+	+	−	
8473	66	M	+	−	−	−	+	+	+	
8482	13	M	+	−	−	+	+	−	++	
8487	48	M	+	−	−	−	+	−	−	
8503	65	M	+	−	−	+	+	+	−	
8562	36	F	+	−	+	+	+	−	−	Also listed under Strep. virid. Gp.
8343	26	F	+	−	+	−	+	−	−	Also listed in Pneumoc. Gp.
8510	37	M	+	−	−	−	+	+	−	
8210	35	M	+	−	−	−	+	−	−	

Chronic Non-Rheumatic Cases

Autopsy No.	Age	Sex	Rheum. Fever	Chorea	Tonsil-litis	Pericar-ditis	Valve Lesions	Scars	Aschoff Bodies	
8240	10	M	−	−	−	−	+	−	−	
8404	65	F	−	−	−	−	+	+	−	
8407	52	F	−	−	−	−	+	−	−	
8417	45	M	−	−	+	−	+	++	−	
8431	54	M	−	−	−	+sl.	+	−	−	
8449	43	F	−	−	−	−	+	−	−	
8466	5	M	−	−	+	−	+	−	−	
8497	45	F	−	−	−	−	+	−	−	
8529	35	M	−	−	++	+	+	+	−	
8539	51	F	−	−	++	−	+	+	−	
8544	59	F	−	−	−	−	+	++	−	

Syphilitic Cases

Autopsy No.	Age	Sex	Rheum. Fever	Chorea	Pericar-ditis	Valve Lesions	Scars	Aschoff Bodies	Wasser. React.	
8439	42	M	+?	−	−	+	++	−	++++	
8453	43	M	−	−	+sl.	+	−	−	++++	
8476	60	M	−	−	+sl.	+	−	−	Not obtained	
8534	33	M	−	−	−	+	++	−	++++	
8549	48	M	−	−	−	+	+	−	+	
8599	46	M	−	−	+sl.	+	++	−	++++	